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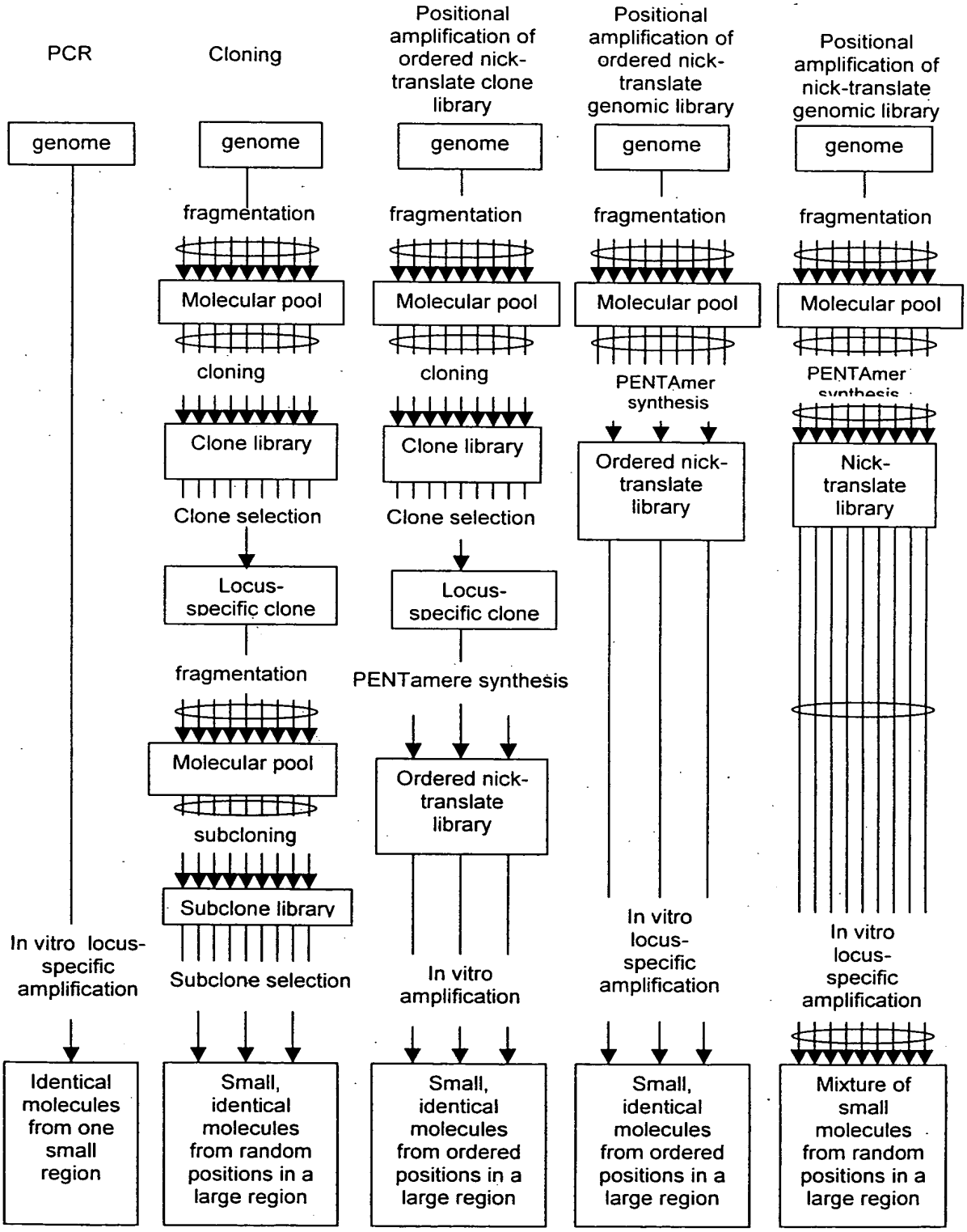

PCR
Cloning
Positional amplification of ordered nick-translate clone library
Positional amplification of ordered nick-translate genomic library
Positional amplification of nick-translate genomic library
genome
fragmentation
Molecular pool
cloning
Clone library
Clone selection
Locus-specific clone
fragmentation
Molecular pool
subcloning
Subclone library
Subclone selection
PENTamere synthesis
Ordered nick-translate library
In vitro amplification
PENTAmer synthesis
Ordered nick-translate library
In vitro locus-specific amplification
Nick-translate library
In vitro locus-specific amplification
In vitro locus-specific amplification
Identical molecules from one small region
Small, identical molecules from random positions in a large region
Small, identical molecules from ordered positions in a large region
Small, identical molecules from ordered positions in a large region
Mixture of small molecules from random positions in a large region

FIG. 1

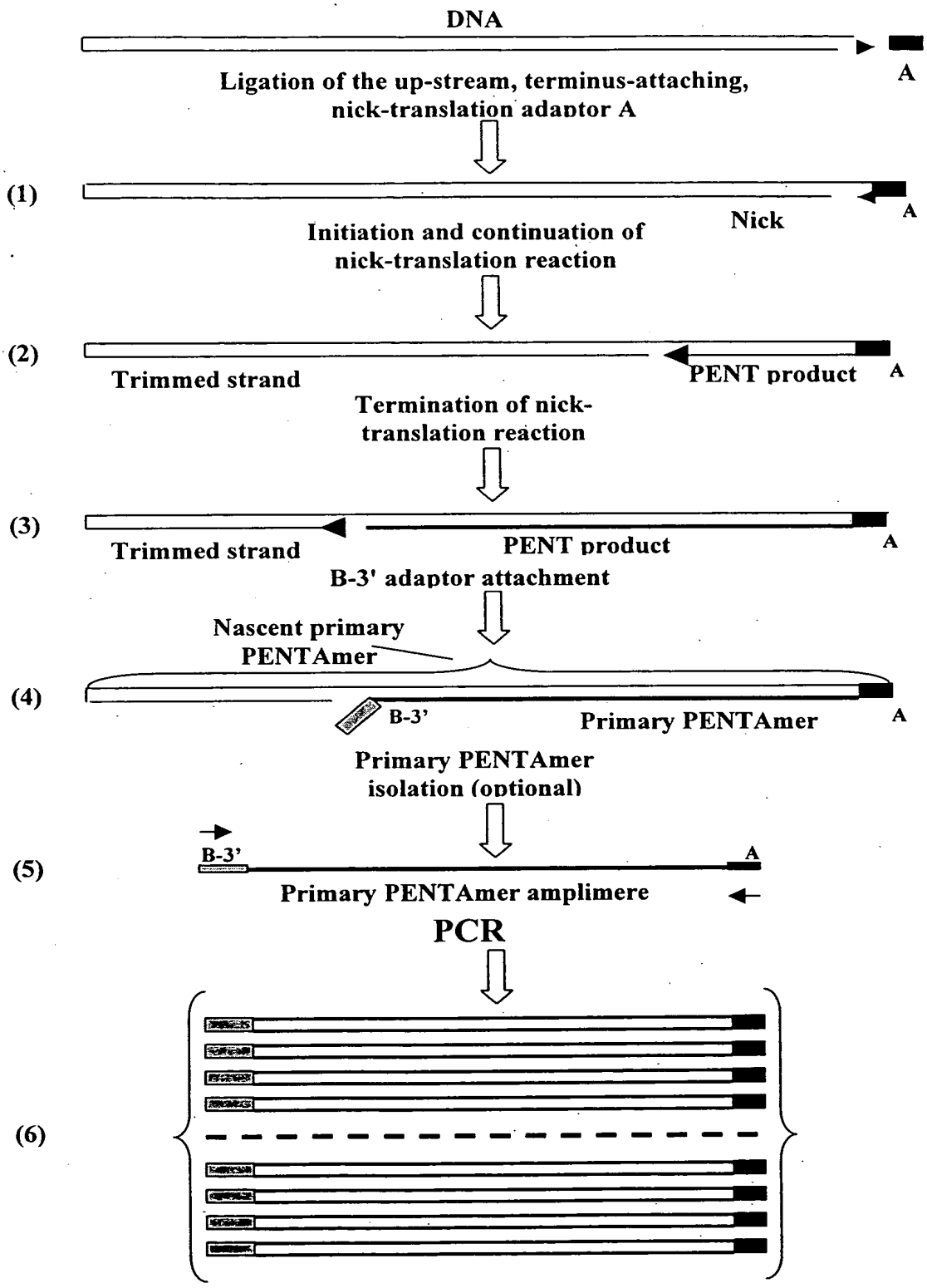

DNA
A
Ligation of the up-stream, terminus-attaching, nick-translation adaptor A
(1)
Nick
A
Initiation and continuation of nick-translation reaction
(2)
Trimmed strand
PENT product
A
Termination of nick-translation reaction
(3)
Trimmed strand
PENT product
A
B-3' adaptor attachment
Nascent primary PENTAmer
(4)
B-3'
Primary PENTAmer
A
Primary PENTAmer isolation (optional)
(5)
B-3'
A
Primary PENTAmer amplimere
PCR
(6)

FIG. 2A

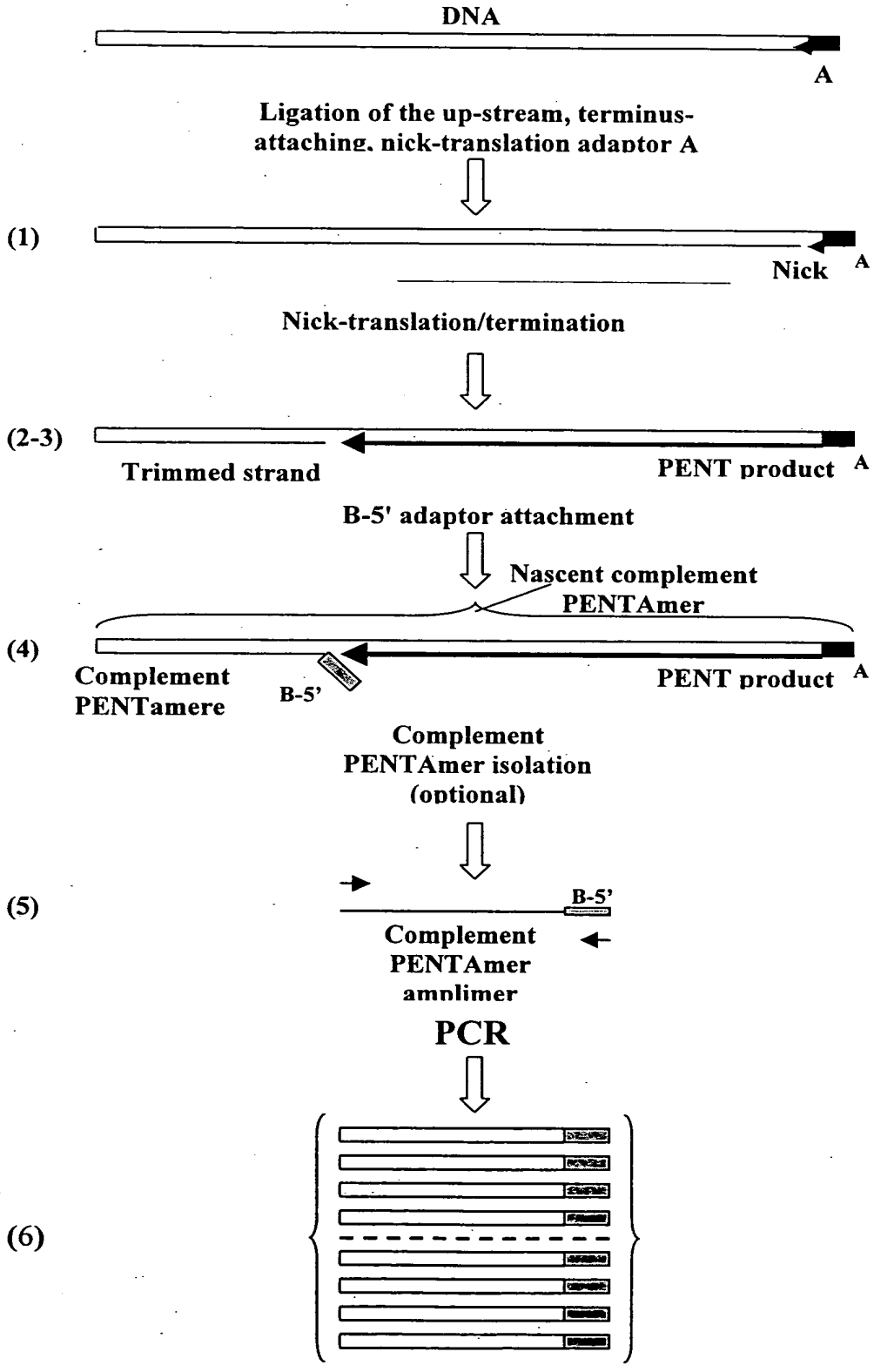

DNA
A
Ligation of the up-stream, terminus-attaching, nick-translation adaptor A
(1)
Nick
A
Nick-translation/termination
(2-3)
Trimmed strand
PENT product
A
B-5' adaptor attachment
Nascent complement PENTAmer
(4)
Complement PENTamere
B-5'
PENT product
A
Complement PENTAmer isolation (optional)
(5)
B-5'
Complement PENTAmer amplimer
PCR
(6)

FIG. 2B

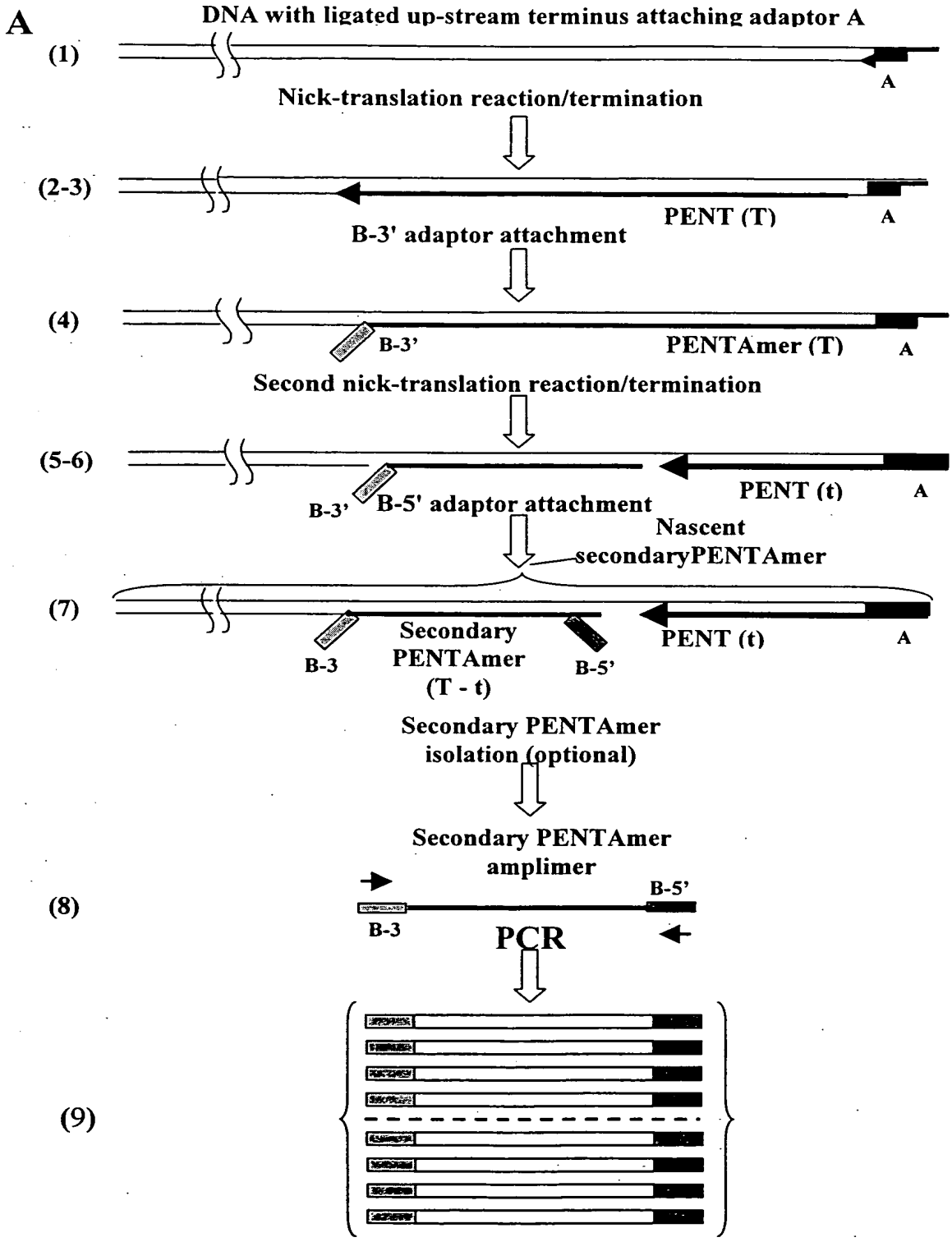

A
DNA with ligated up-stream terminus attaching adaptor A
(1)
A
Nick-translation reaction/termination
(2-3)
PENT (T)
A
B-3' adaptor attachment
(4)
B-3'
PENTAmer (T)
A
Second nick-translation reaction/termination
(5-6)
B-3'
B-5' adaptor attachment
PENT (t)
A
Nascent
secondaryPENTAmer
(7)
B-3
Secondary
PENTAmer
(T - t)
B-5'
PENT (t)
A
Secondary PENTAmer
isolation (optional)
Secondary PENTAmer
amplimer
(8)
B-5'
B-3
PCR
(9)

FIG. 3A

B

(1 - 3) as in FIG. 3A

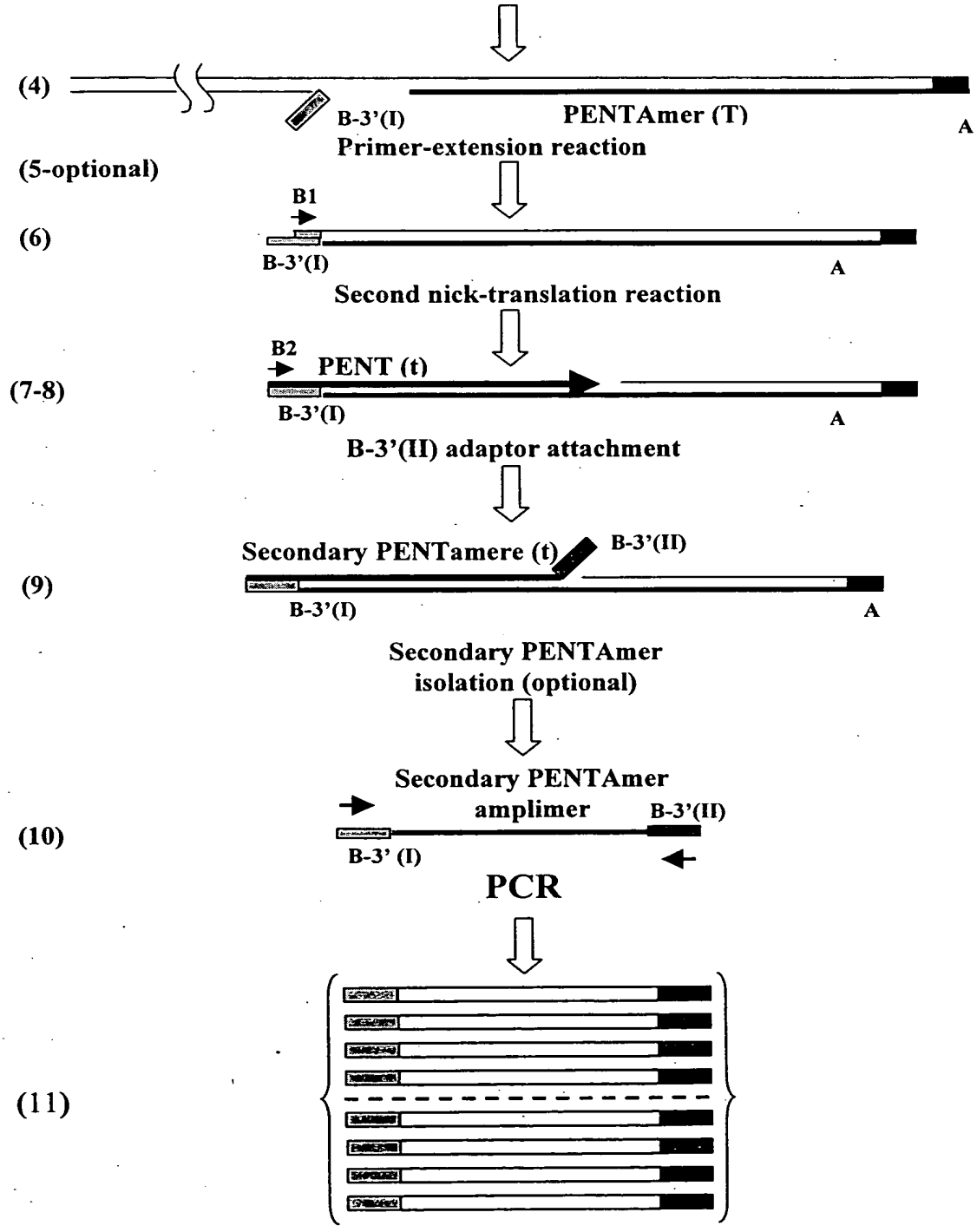


FIG. 3B

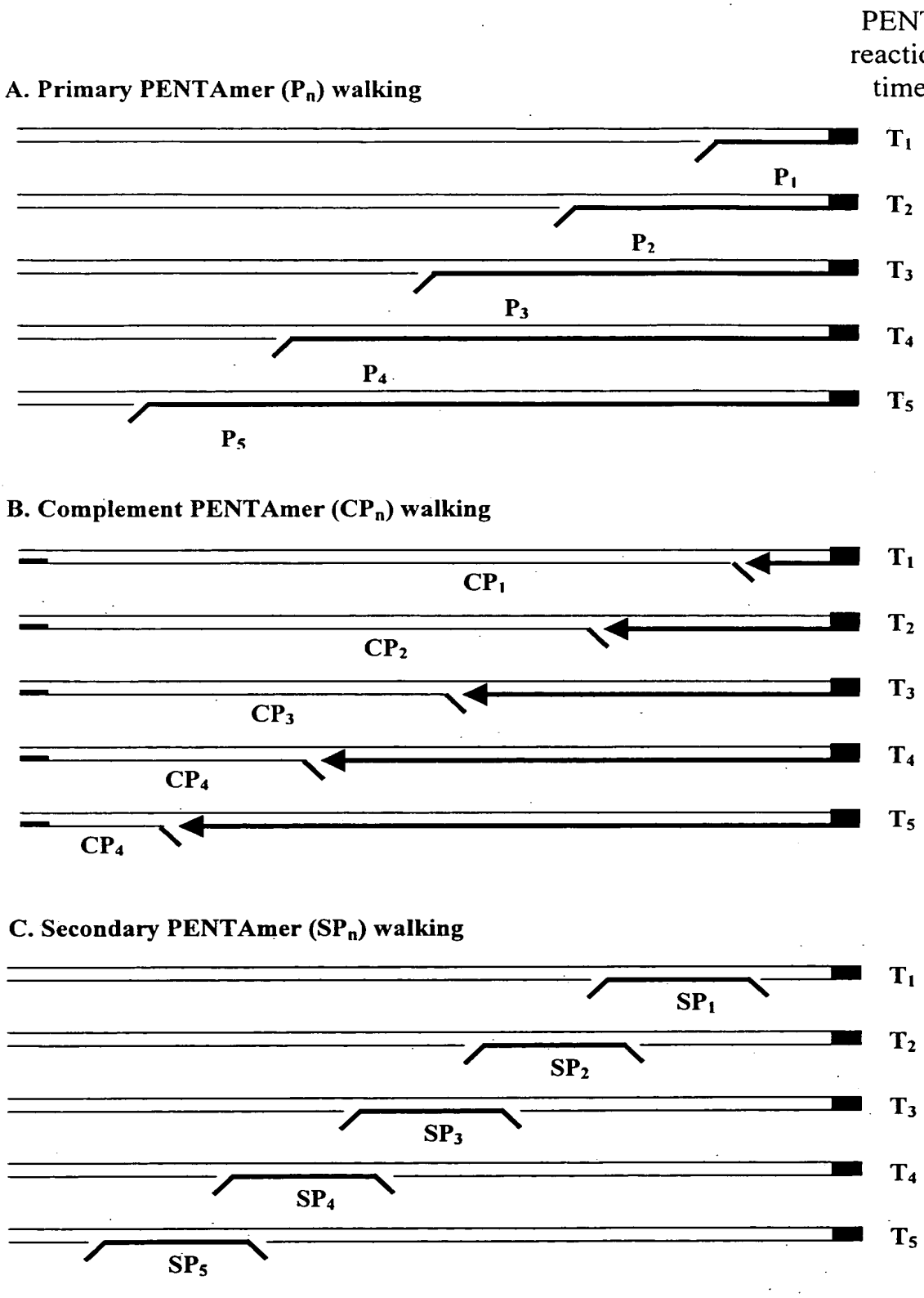

PENT reaction time
A. Primary PENTAmer (P_n) walking
T_1
P_1
T_2
P_2
T_3
P_3
T_4
P_4
T_5
P_5
B. Complement PENTAmer (CP_n) walking
T_1
CP_1
T_2
CP_2
T_3
CP_3
T_4
CP_4
T_5
CP_4
C. Secondary PENTAmer (SP_n) walking
T_1
SP_1
T_2
SP_2
T_3
SP_3
T_4
SP_4
T_5
SP_5

FIG. 4

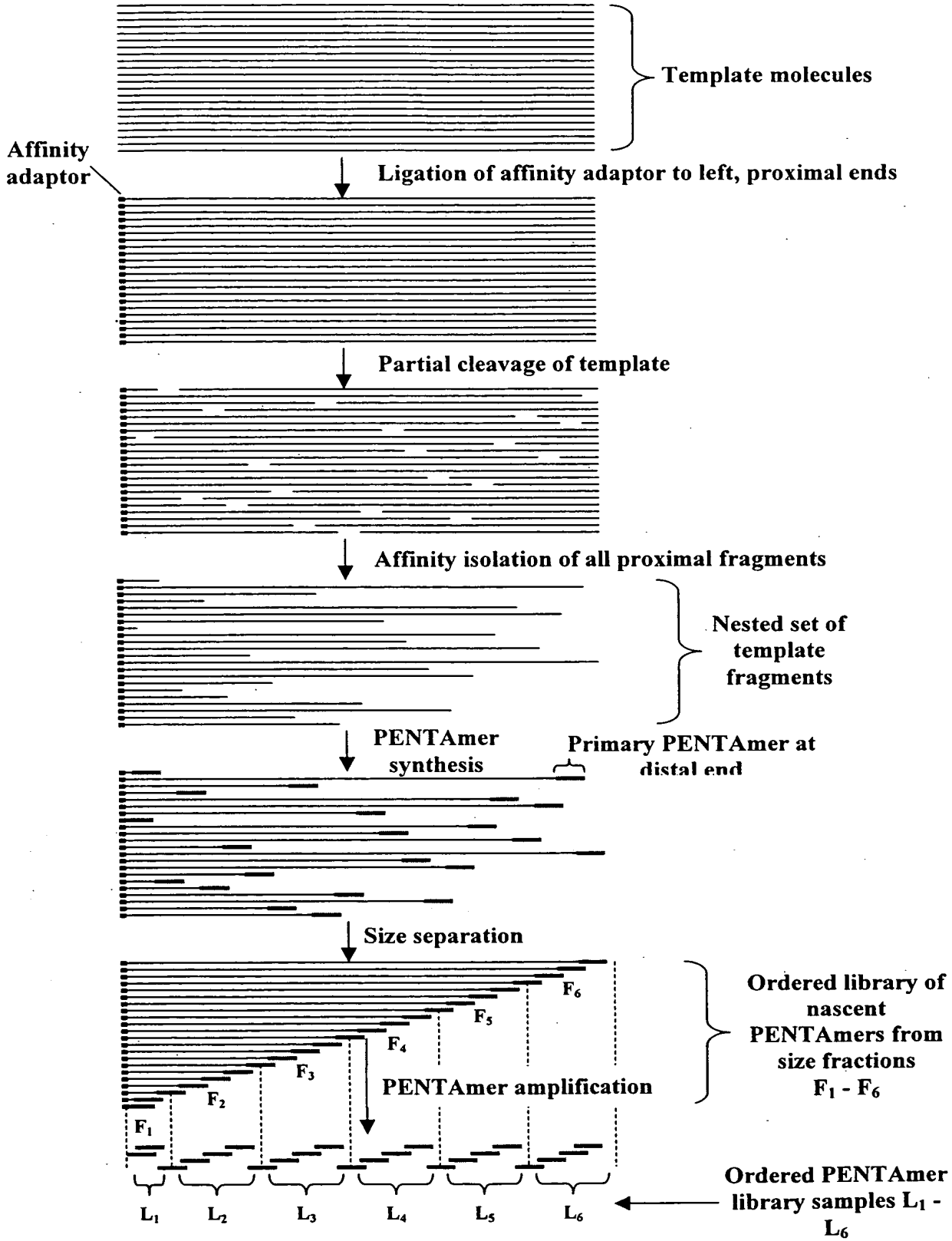

Template molecules
Affinity adaptor
Ligation of affinity adaptor to left, proximal ends
Partial cleavage of template
Affinity isolation of all proximal fragments
Nested set of template fragments
PENTAmer synthesis
Primary PENTAmer at distal end
Size separation
F6
F5
F4
F3
F2
F1
PENTAmer amplification
Ordered library of nascent PENTAmers from size fractions F1 - F6
L1
L2
L3
L4
L5
L6
Ordered PENTAmer library samples L1 - L6

FIG. 5

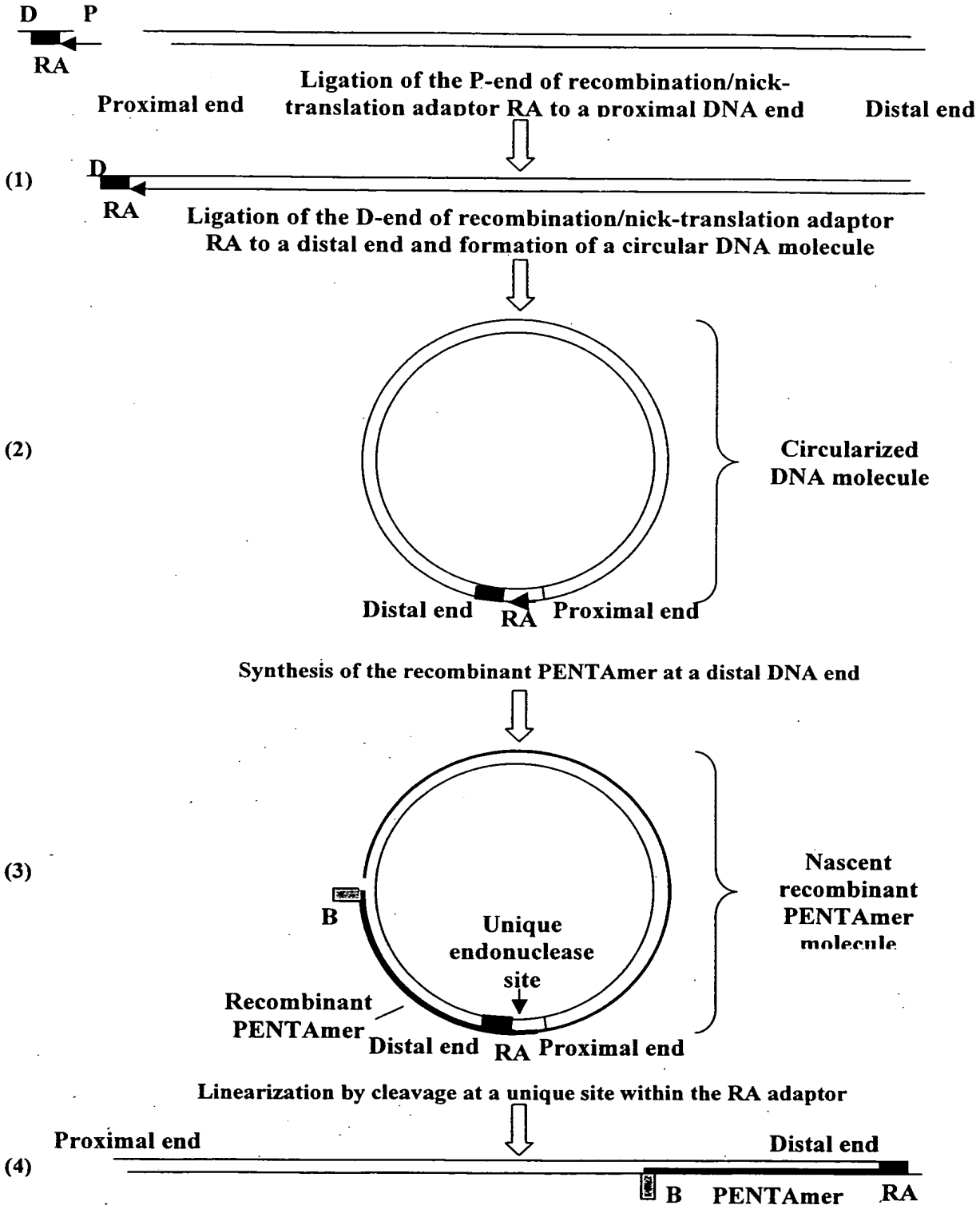

D
P
RA
Proximal end
Ligation of the P-end of recombination/nick-translation adaptor RA to a proximal DNA end
Distal end
(1)
D
RA
Ligation of the D-end of recombination/nick-translation adaptor RA to a distal end and formation of a circular DNA molecule
(2)
Circularized DNA molecule
Distal end
RA
Proximal end
Synthesis of the recombinant PENTAmer at a distal DNA end
(3)
B
Unique endonuclease site
Nascent recombinant PENTAmer molecule
Recombinant PENTAmer
Distal end
RA
Proximal end
Linearization by cleavage at a unique site within the RA adaptor
Proximal end
Distal end
(4)
B
PENTAmer
RA

FIG. 6

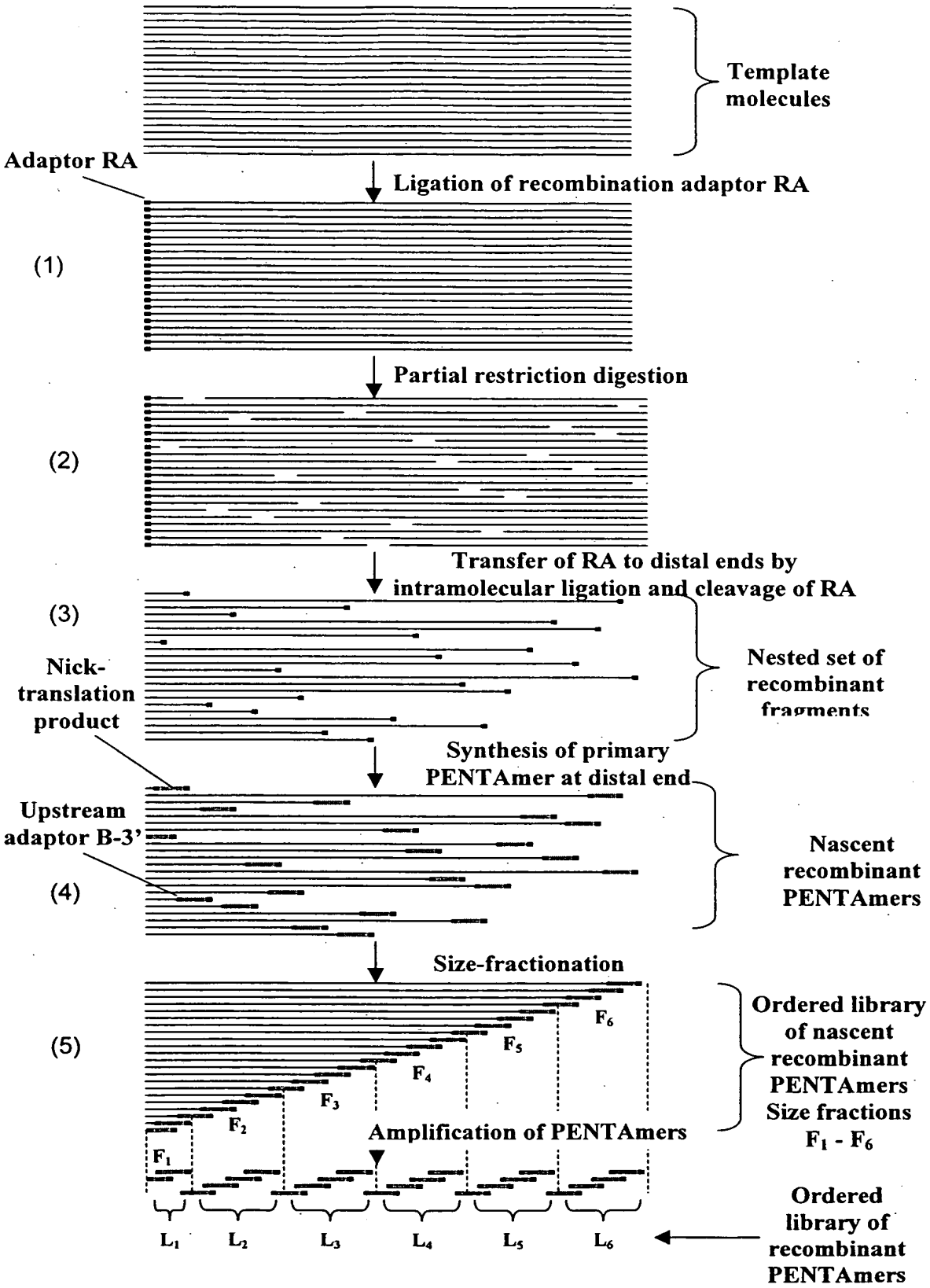

Template molecules
Adaptor RA
Ligation of recombination adaptor RA
(1)
Partial restriction digestion
(2)
Transfer of RA to distal ends by intramolecular ligation and cleavage of RA
(3)
Nick-translation product
Nested set of recombinant fragments
Synthesis of primary PENTAmer at distal end
Upstream adaptor B-3'
(4)
Nascent recombinant PENTAmers
Size-fractionation
(5)
F1
F2
F3
F4
F5
F6
Ordered library of nascent recombinant PENTAmers Size fractions F1 - F6
Amplification of PENTAmers
L1
L2
L3
L4
L5
L6
Ordered library of recombinant PENTAmers

FIG. 7

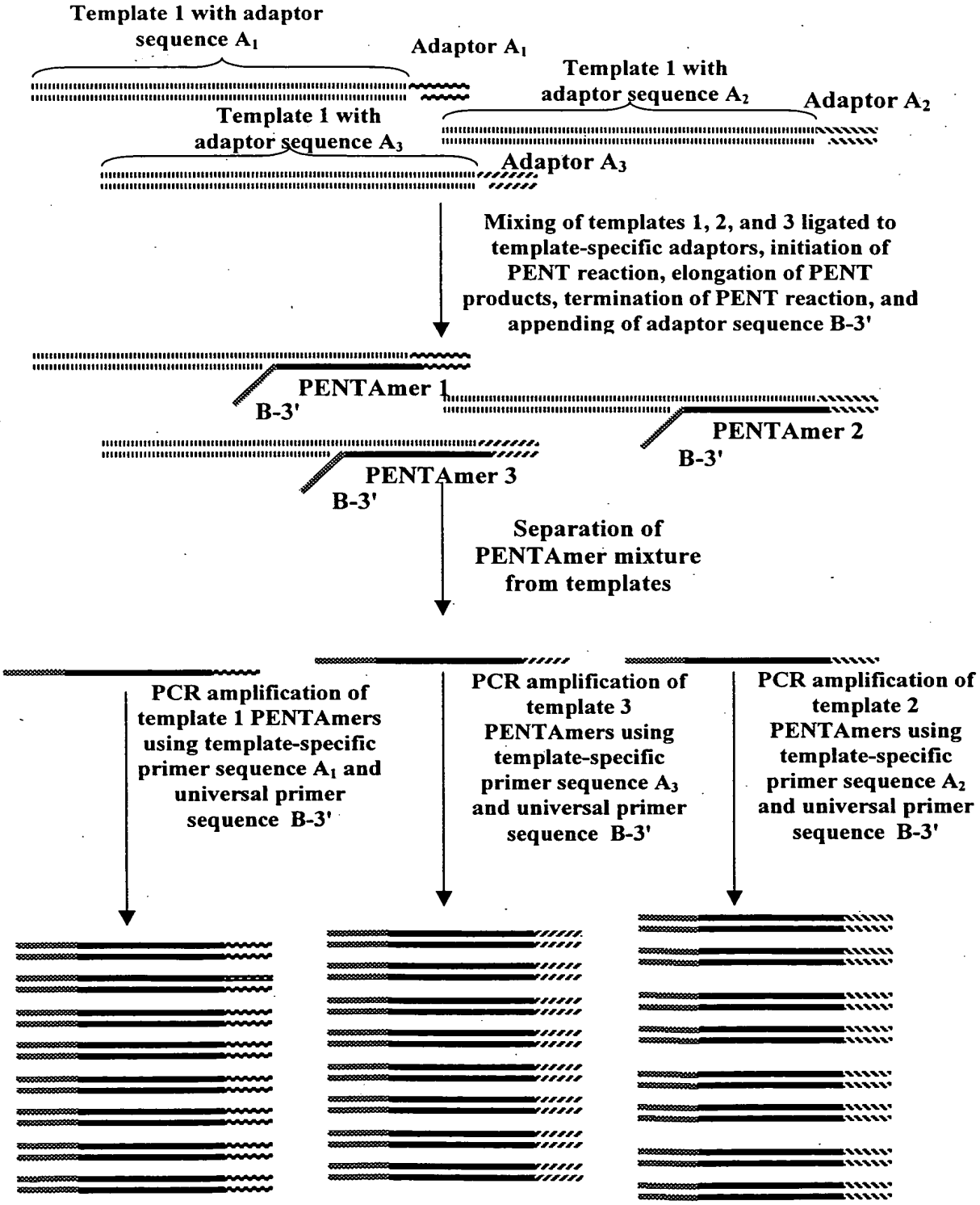

Template 1 with adaptor sequence A_1
Adaptor A_1
Template 1 with adaptor sequence A_2
Adaptor A_2
Template 1 with adaptor sequence A_3
Adaptor A_3
Mixing of templates 1, 2, and 3 ligated to template-specific adaptors, initiation of PENT reaction, elongation of PENT products, termination of PENT reaction, and appending of adaptor sequence B-3'
PENTAmer 1
B-3'
PENTAmer 2
B-3'
PENTAmer 3
B-3'
Separation of PENTAmer mixture from templates
PCR amplification of template 1 PENTAmers using template-specific primer sequence A_1 and universal primer sequence B-3'
PCR amplification of template 3 PENTAmers using template-specific primer sequence A_3 and universal primer sequence B-3'
PCR amplification of template 2 PENTAmers using template-specific primer sequence A_2 and universal primer sequence B-3'

FIG. 8

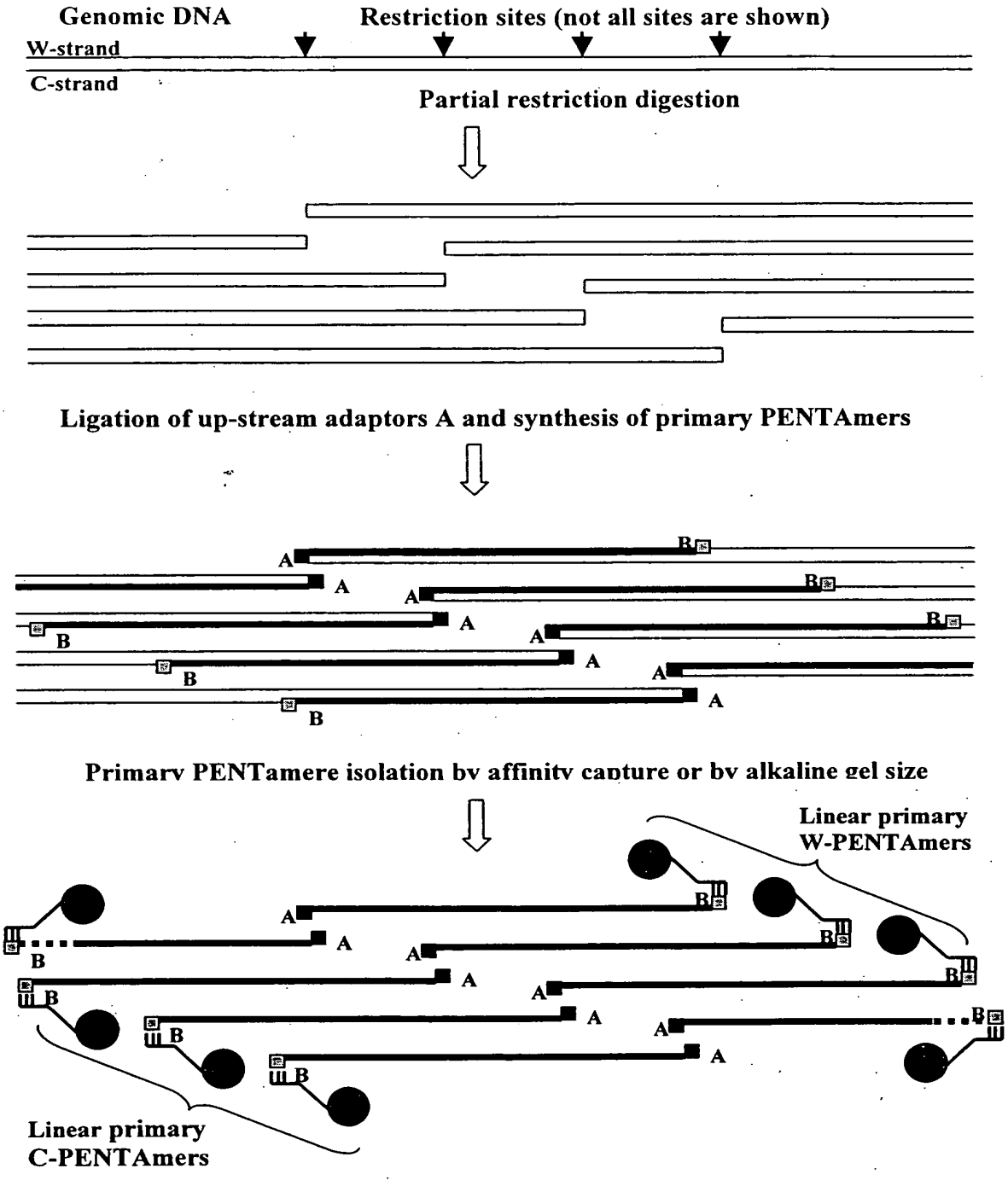

Genomic DNA
Restriction sites (not all sites are shown)
W-strand
C-strand
Partial restriction digestion
Ligation of up-stream adaptors A and synthesis of primary PENTAmers
Primary PENTamers isolation by affinity capture or by alkaline gel size
Linear primary
W-PENTAmers
Linear primary
C-PENTAmers

FIG. 9A

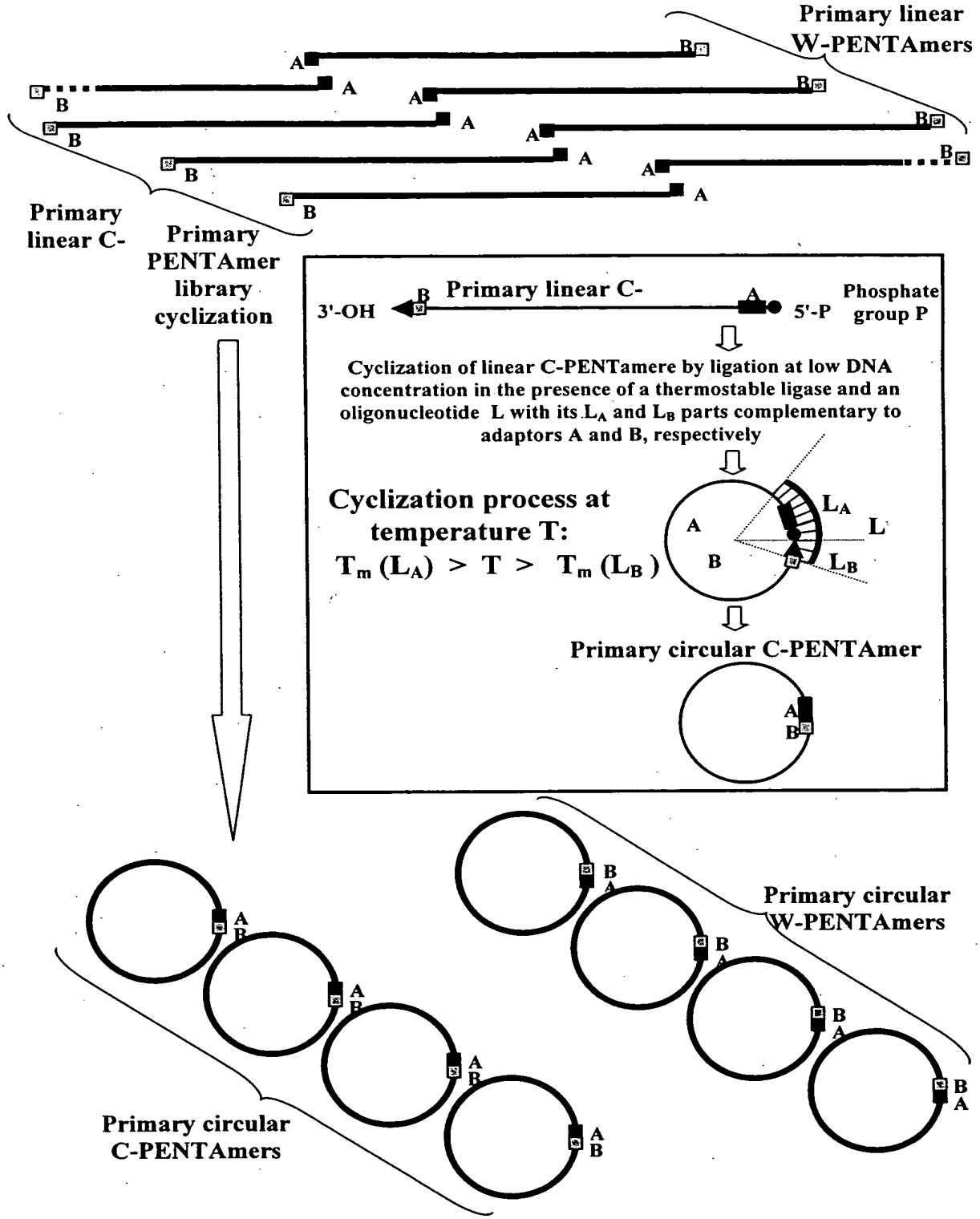

Primary linear W-PENTAmers
Primary linear C-
Primary PENTAmer library cyclization
3'-OH
Primary linear C-
5'-P
Phosphate group P
Cyclization of linear C-PENTamere by ligation at low DNA concentration in the presence of a thermostable ligase and an oligonucleotide L with its L_A and L_B parts complementary to adaptors A and B, respectively
Cyclization process at temperature T:
$T_m (L_A) > T > T_m (L_B)$
L_A
L
L_B
Primary circular C-PENTAmer
Primary circular W-PENTAmers
Primary circular C-PENTAmers

FIG. 9B

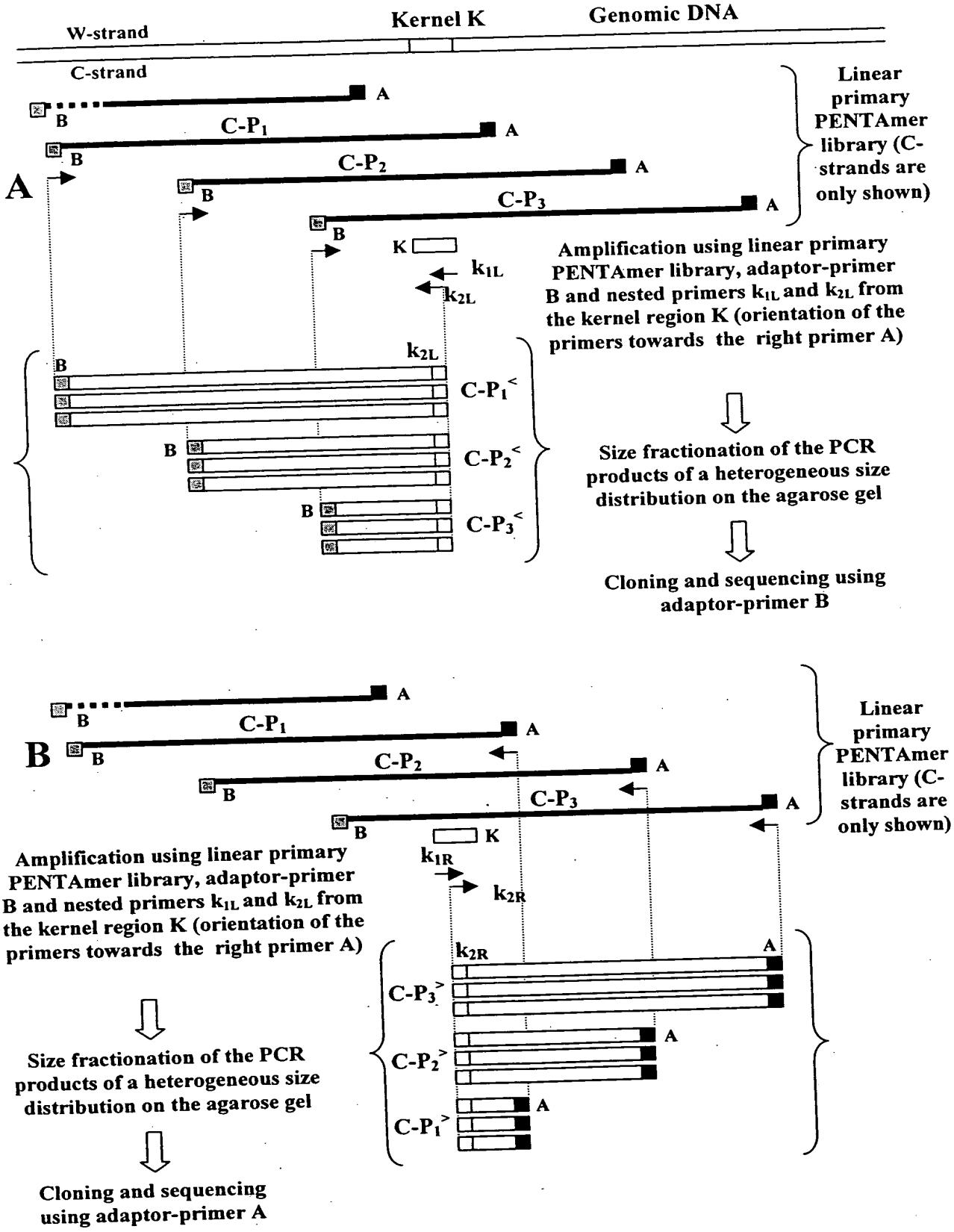

W-strand
Kernel K
Genomic DNA
C-strand
A
B
C-P1
C-P2
C-P3
Linear primary PENTAmer library (C-strands are only shown)
K
k1L
k2L
Amplification using linear primary PENTAmer library, adaptor-primer B and nested primers k1L and k2L from the kernel region K (orientation of the primers towards the right primer A)
C-P1<
C-P2<
C-P3<
Size fractionation of the PCR products of a heterogeneous size distribution on the agarose gel
Cloning and sequencing using adaptor-primer B
Linear primary PENTAmer library (C-strands are only shown)
k1R
k2R
Amplification using linear primary PENTAmer library, adaptor-primer B and nested primers k1L and k2L from the kernel region K (orientation of the primers towards the right primer A)
C-P3>
C-P2>
C-P1>
Size fractionation of the PCR products of a heterogeneous size distribution on the agarose gel
Cloning and sequencing using adaptor-primer A

FIG. 10

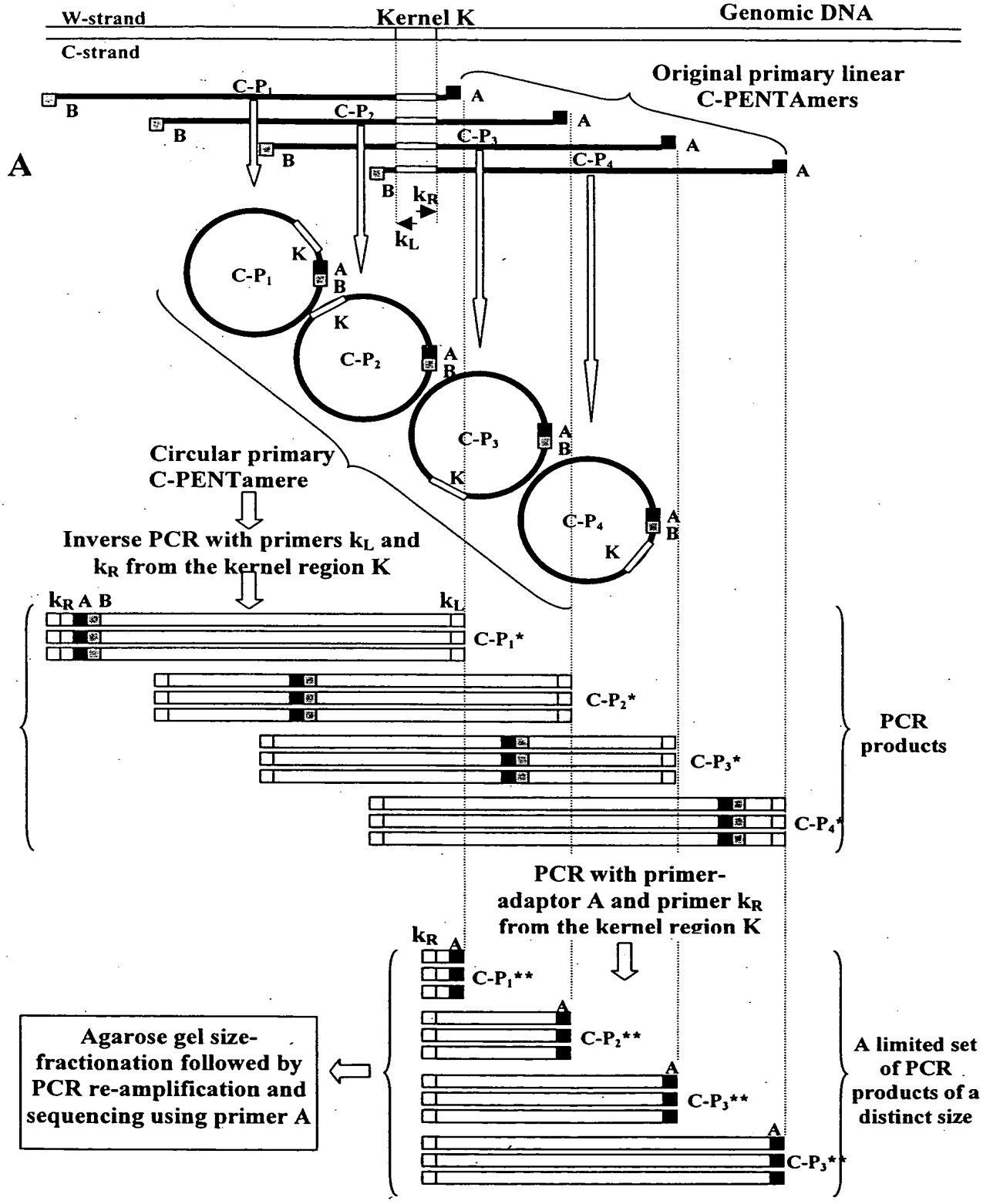

A
W-strand
C-strand
Kernel K
Genomic DNA
Original primary linear C-PENTAmers
C-P1
C-P2
C-P3
C-P4
A
B
kR
kL
K
Circular primary C-PENTamere
Inverse PCR with primers kL and kR from the kernel region K
kR A B
kL
C-P1*
C-P2*
C-P3*
C-P4*
PCR products
PCR with primer-adaptor A and primer kR from the kernel region K
C-P1**
C-P2**
C-P3**
C-P3**
A limited set of PCR products of a distinct size
Agarose gel size-fractionation followed by PCR re-amplification and sequencing using primer A

FIG. 11A

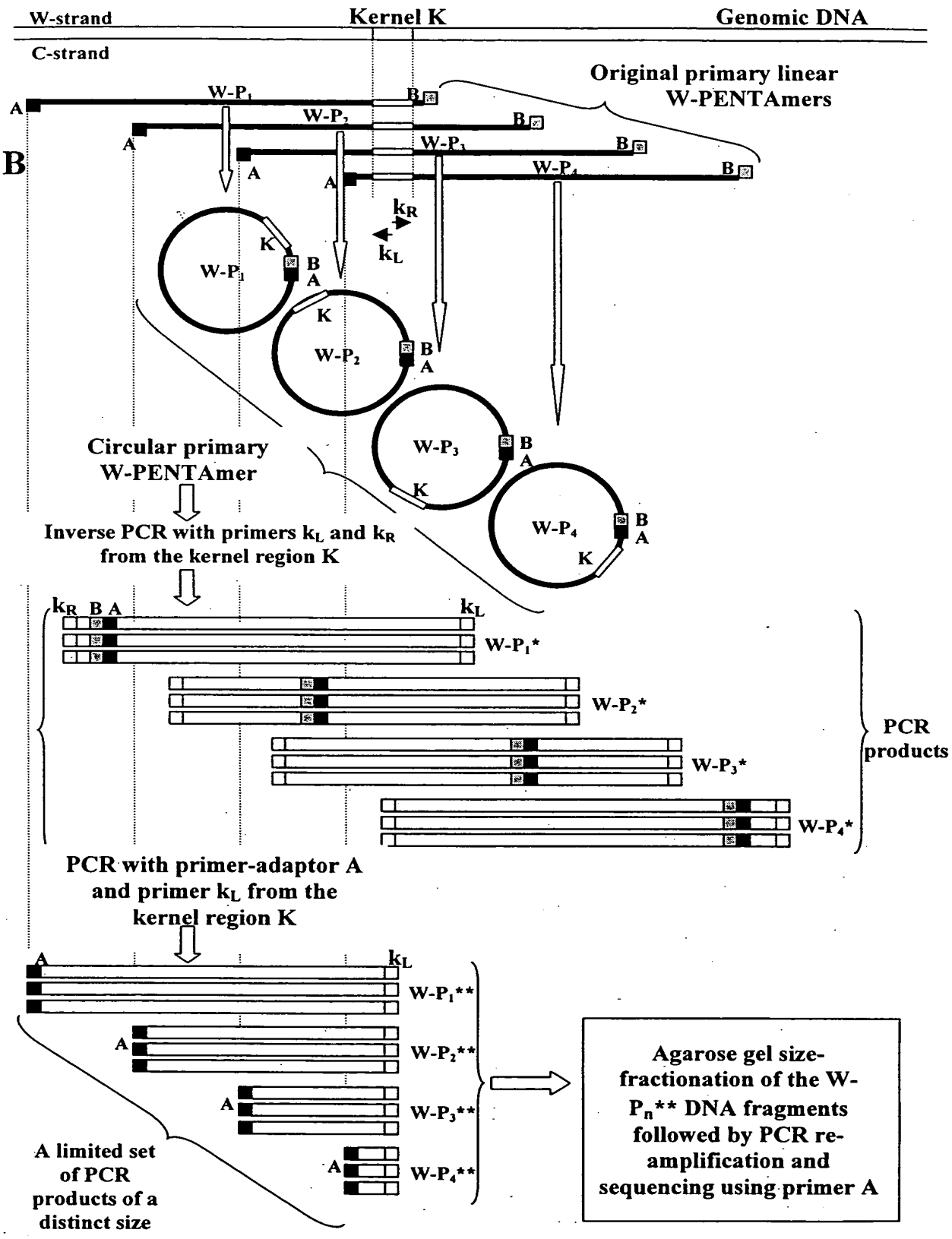

W-strand
Kernel K
Genomic DNA
C-strand
Original primary linear W-PENTAmers
Circular primary W-PENTAmer
Inverse PCR with primers k_L and k_R from the kernel region K
PCR products
PCR with primer-adaptor A and primer k_L from the kernel region K
A limited set of PCR products of a distinct size
Agarose gel size-fractionation of the W-P_n** DNA fragments followed by PCR re-amplification and sequencing using primer A

FIG. 11B

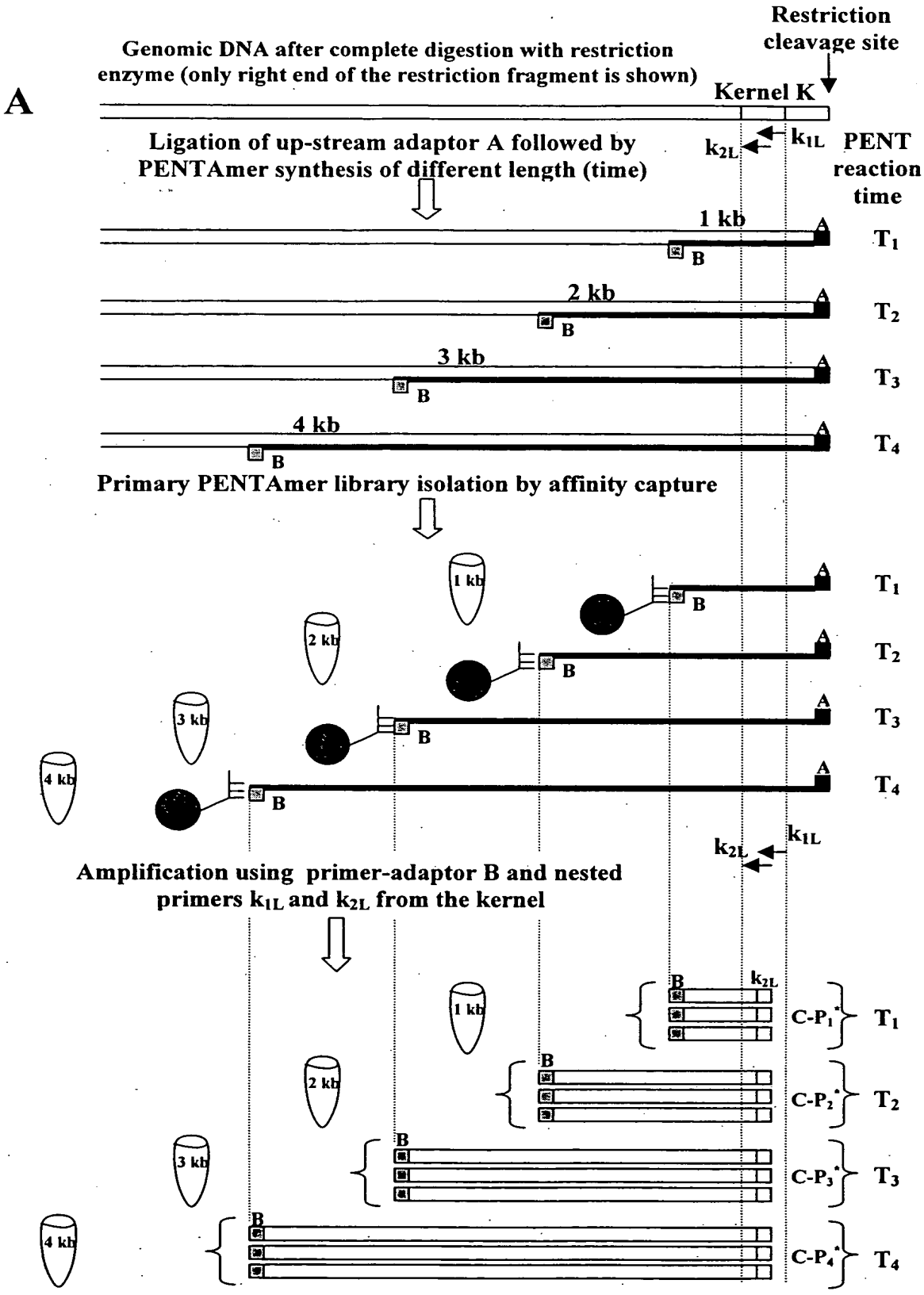

A
Genomic DNA after complete digestion with restriction enzyme (only right end of the restriction fragment is shown)
Restriction cleavage site
Kernel K
k_{2L}
k_{1L}
Ligation of up-stream adaptor A followed by PENTAmer synthesis of different length (time)
PENT reaction time
1 kb
2 kb
3 kb
4 kb
B
T_1
T_2
T_3
T_4
Primary PENTAmer library isolation by affinity capture
Amplification using primer-adaptor B and nested primers k_{1L} and k_{2L} from the kernel
$C\text{-}P_1^*$
$C\text{-}P_2^*$
$C\text{-}P_3^*$
$C\text{-}P_4^*$

FIG. 12A

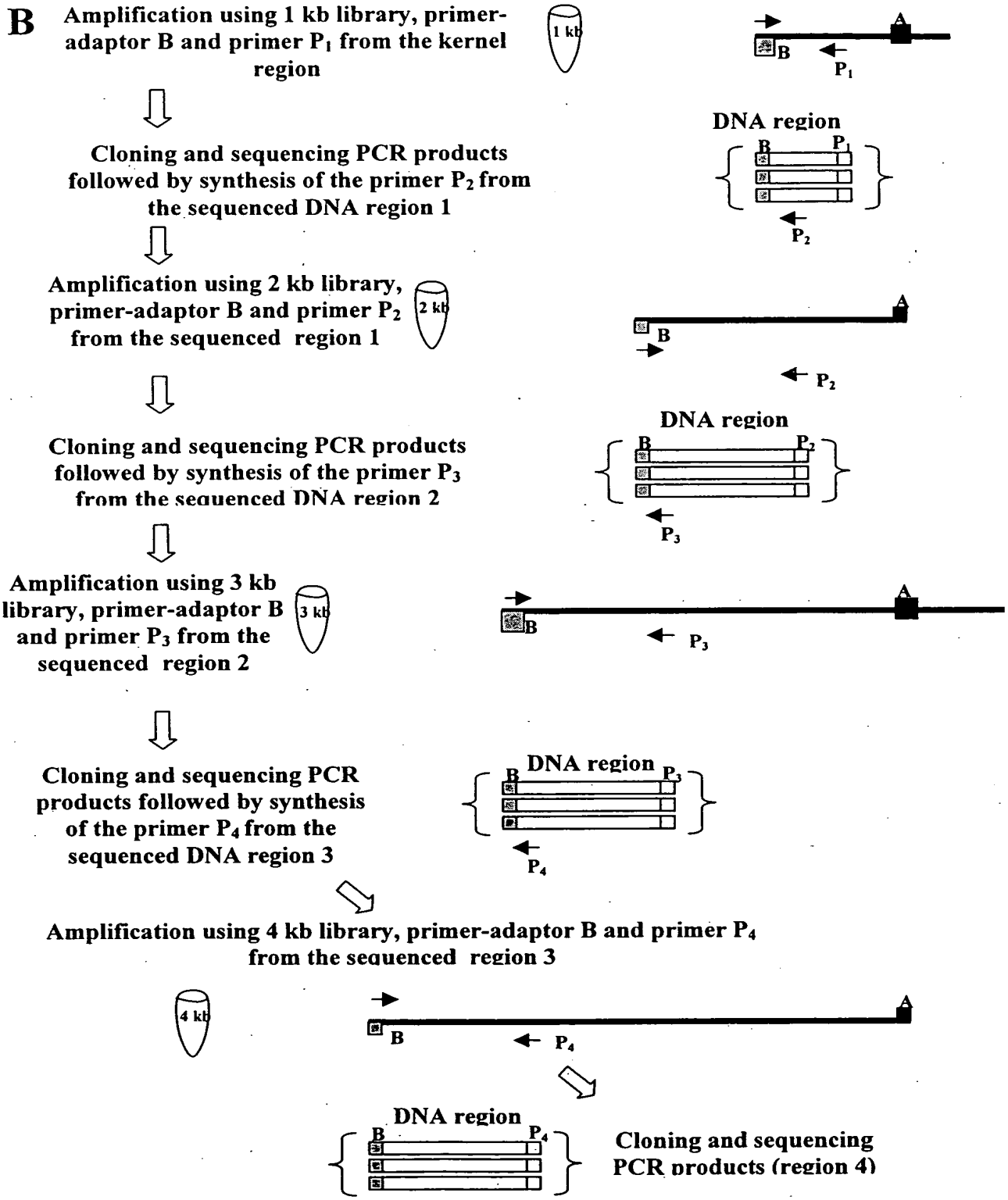

B
Amplification using 1 kb library, primer-adaptor B and primer P1 from the kernel region
1 kb
A
B
P1
DNA region
B
P1
P2
Cloning and sequencing PCR products followed by synthesis of the primer P2 from the sequenced DNA region 1
Amplification using 2 kb library, primer-adaptor B and primer P2 from the sequenced region 1
2 kb
A
B
P2
DNA region
B
P2
P3
Cloning and sequencing PCR products followed by synthesis of the primer P3 from the sequenced DNA region 2
Amplification using 3 kb library, primer-adaptor B and primer P3 from the sequenced region 2
3 kb
A
B
P3
DNA region
B
P3
P4
Cloning and sequencing PCR products followed by synthesis of the primer P4 from the sequenced DNA region 3
Amplification using 4 kb library, primer-adaptor B and primer P4 from the sequenced region 3
4 kb
A
B
P4
DNA region
B
P4
Cloning and sequencing PCR products (region 4)

FIG. 12B

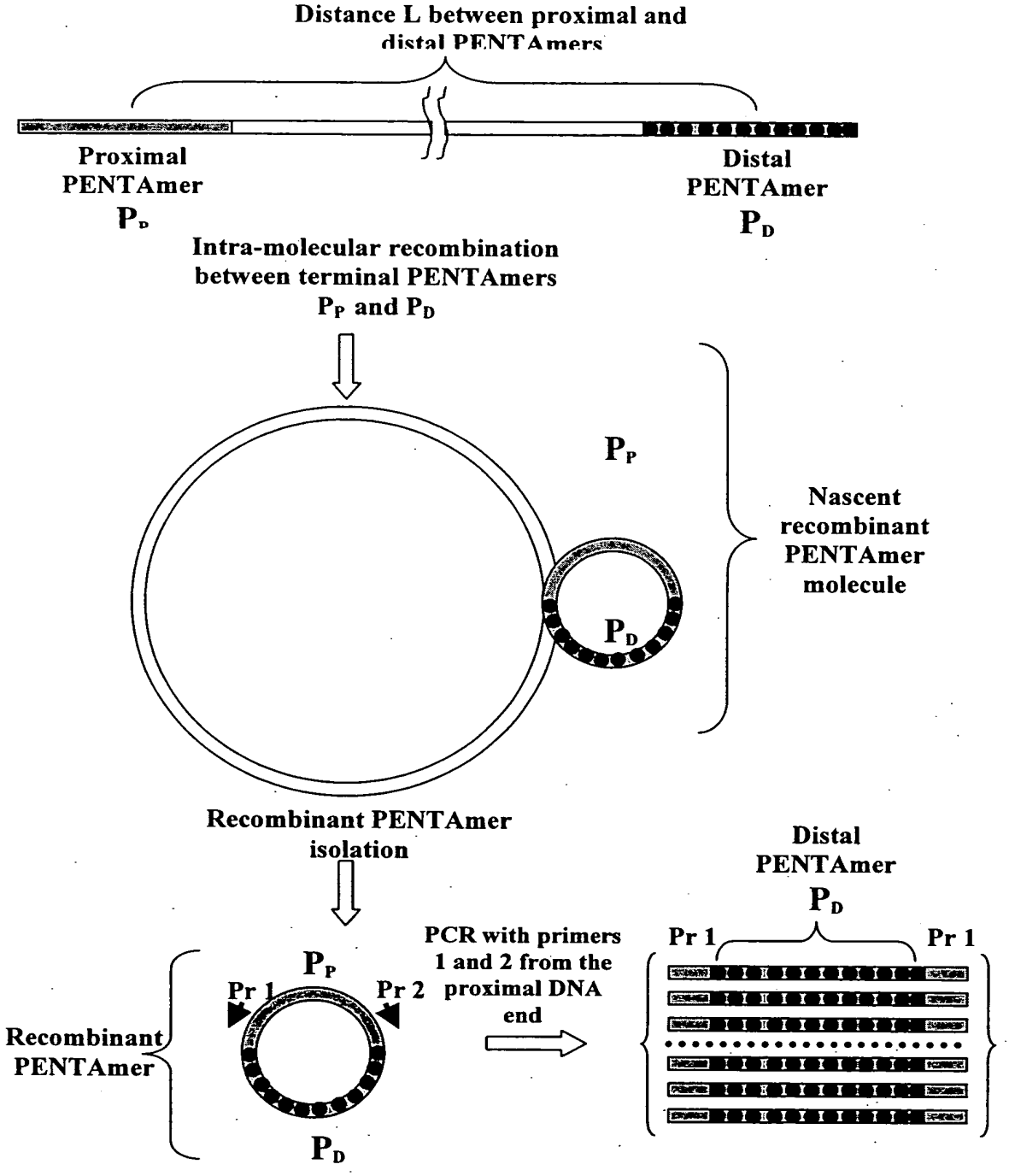

Distance L between proximal and distal PENTAmers
Proximal PENTAmer
P_P
Distal PENTAmer
P_D
Intra-molecular recombination between terminal PENTAmers P_P and P_D
P_P
P_D
Nascent recombinant PENTAmer molecule
Recombinant PENTAmer isolation
Distal PENTAmer
P_D
PCR with primers 1 and 2 from the proximal DNA end
Pr 1
Pr 1
Recombinant PENTAmer
P_P
Pr 1
Pr 2
P_D

FIG. 13

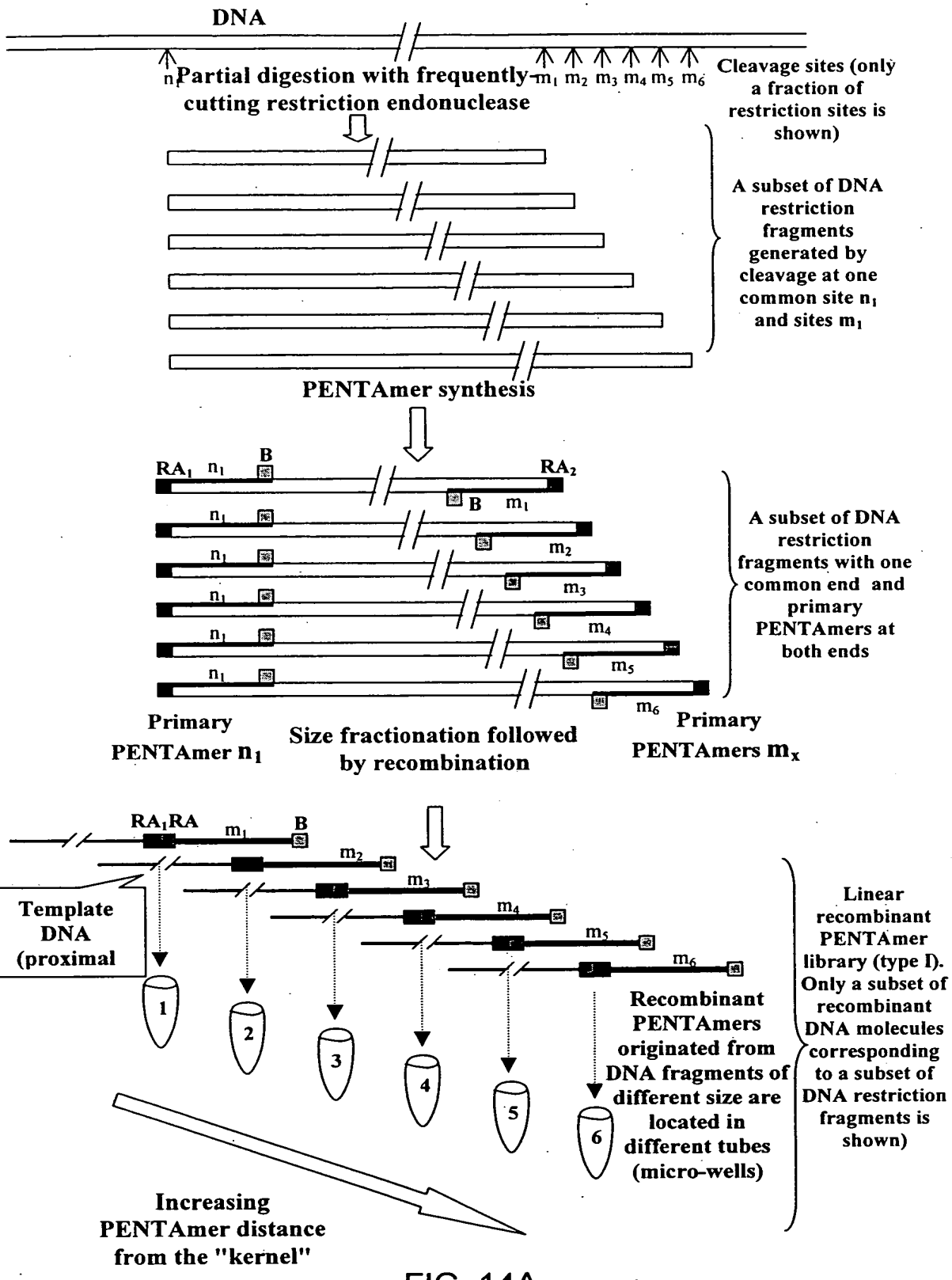

DNA
n1
Partial digestion with frequently cutting restriction endonuclease
m1 m2 m3 m4 m5 m6
Cleavage sites (only a fraction of restriction sites is shown)
A subset of DNA restriction fragments generated by cleavage at one common site n1 and sites m1
PENTAmer synthesis
RA1 n1 B
RA2
B m1
m2
m3
m4
m5
m6
A subset of DNA restriction fragments with one common end and primary PENTAmers at both ends
Primary PENTAmer n1
Size fractionation followed by recombination
Primary PENTAmers mx
RA1RA
Template DNA (proximal
1
2
3
4
5
6
Recombinant PENTAmers originated from DNA fragments of different size are located in different tubes (micro-wells)
Linear recombinant PENTAmer library (type I). Only a subset of recombinant DNA molecules corresponding to a subset of DNA restriction fragments is shown)
Increasing PENTAmer distance from the "kernel"

FIG. 14A

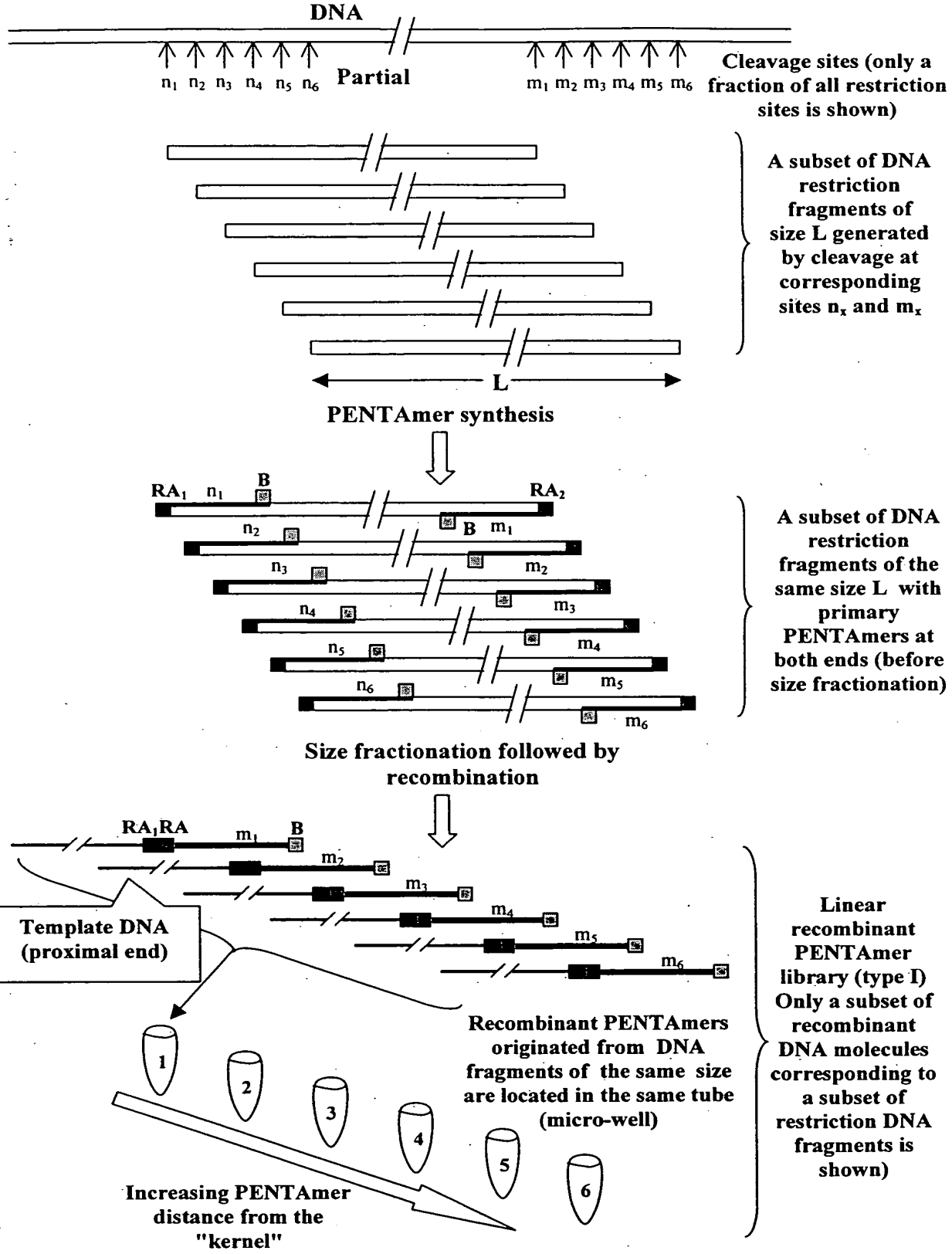

DNA
Partial
Cleavage sites (only a fraction of all restriction sites is shown)
A subset of DNA restriction fragments of size L generated by cleavage at corresponding sites n_x and m_x
L
PENTAmer synthesis
A subset of DNA restriction fragments of the same size L with primary PENTAmers at both ends (before size fractionation)
Size fractionation followed by recombination
Template DNA (proximal end)
Recombinant PENTAmers originated from DNA fragments of the same size are located in the same tube (micro-well)
Increasing PENTAmer distance from the "kernel"
Linear recombinant PENTAmer library (type I) Only a subset of recombinant DNA molecules corresponding to a subset of restriction DNA fragments is shown)

FIG. 14B

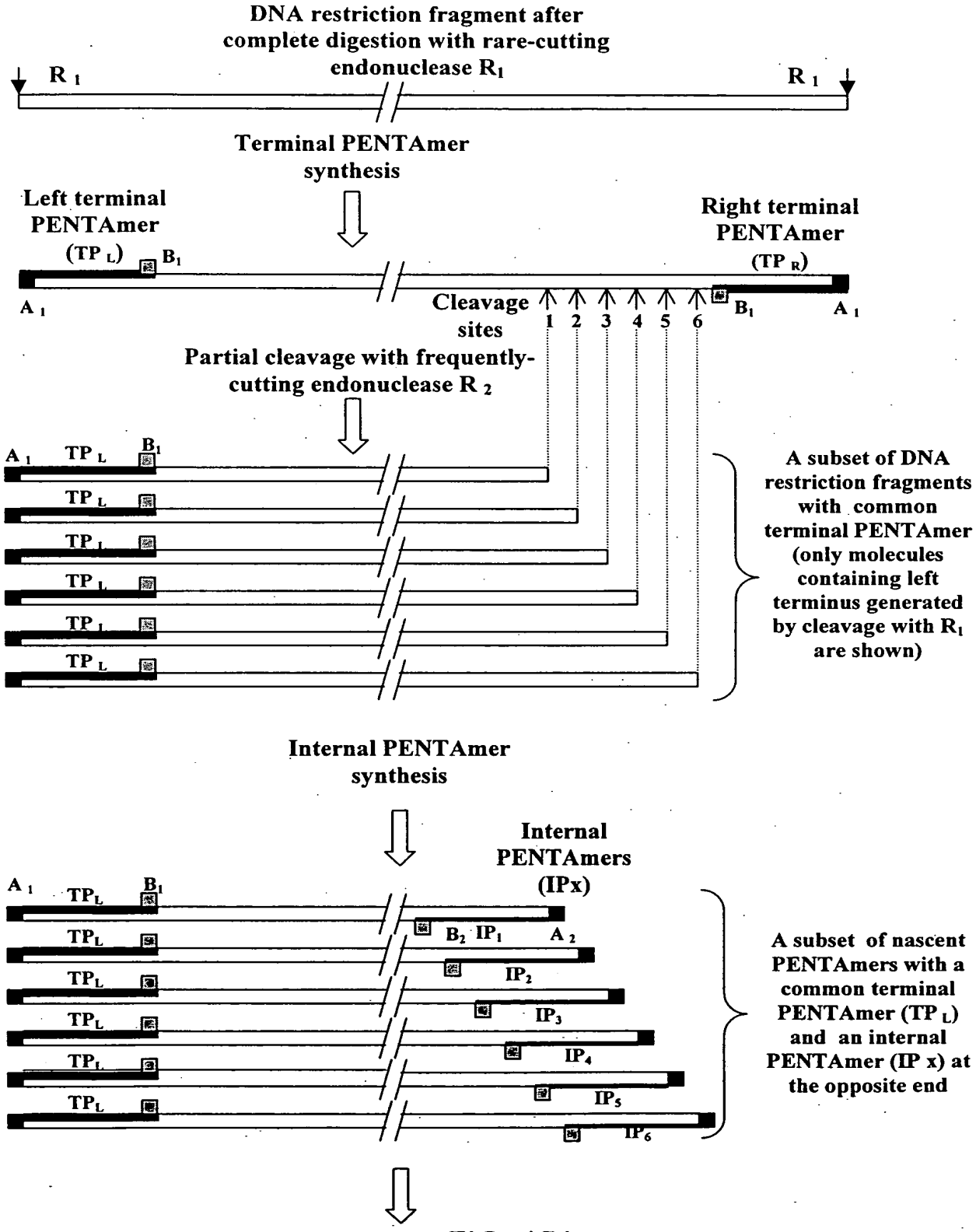

DNA restriction fragment after complete digestion with rare-cutting endonuclease R1
R 1
R 1
Terminal PENTAmer synthesis
Left terminal PENTAmer (TP L)
B1
Right terminal PENTAmer (TP R)
A 1
Cleavage sites
1 2 3 4 5 6
B1
A 1
Partial cleavage with frequently-cutting endonuclease R 2
A 1 TP L B1
TP L
TP L
TP L
TP L
TP L
A subset of DNA restriction fragments with common terminal PENTAmer (only molecules containing left terminus generated by cleavage with R1 are shown)
Internal PENTAmer synthesis
Internal PENTAmers (IPx)
A 1 TPL B1
TPL
TPL
TPL
TPL
TPL
B2 IP1 A 2
IP2
IP3
IP4
IP5
IP6
A subset of nascent PENTAmers with a common terminal PENTAmer (TP L) and an internal PENTAmer (IP x) at the opposite end

FIG. 15A

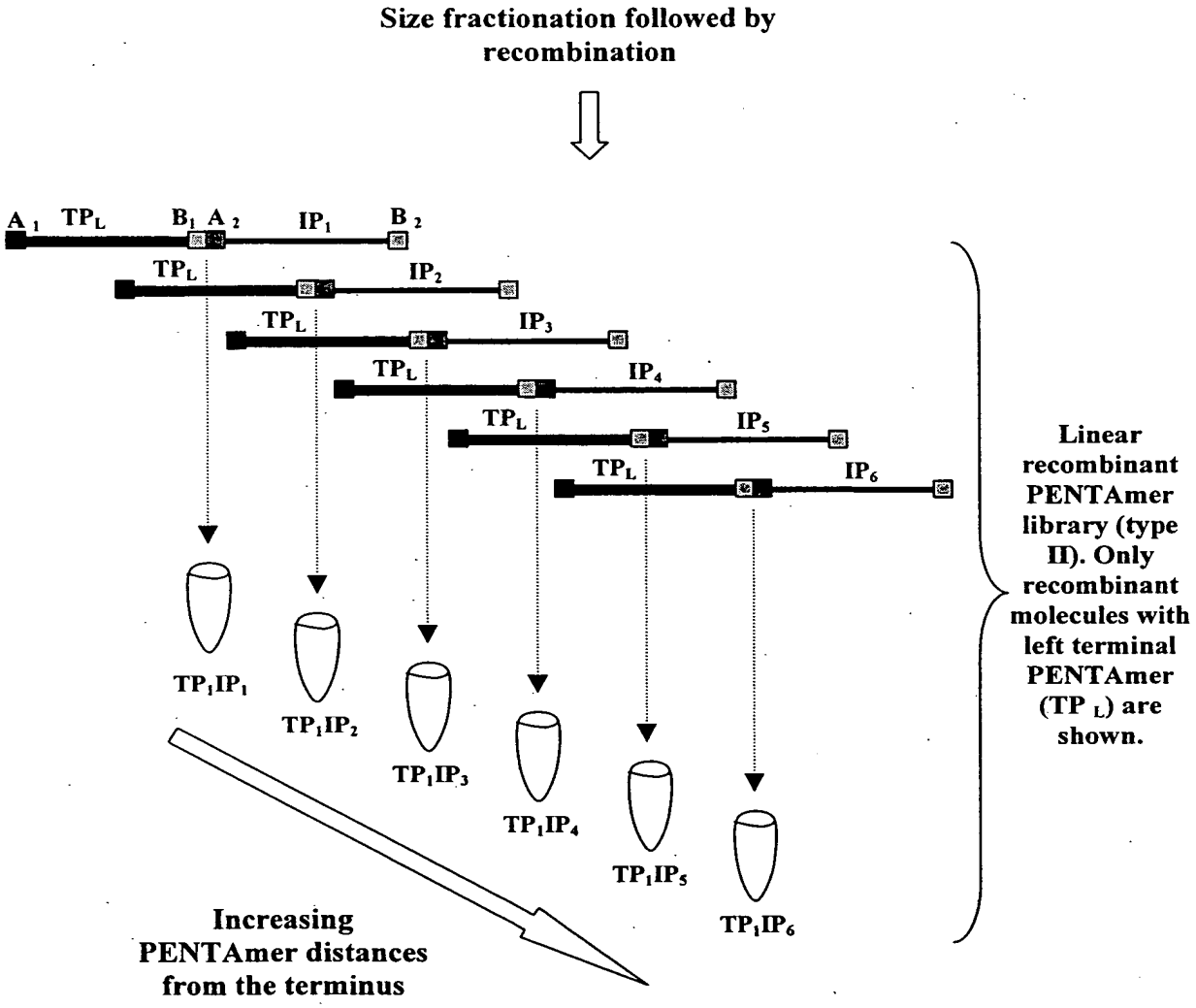

Size fractionation followed by recombination
A 1 TPL B1 A 2 IP1 B 2
TPL IP2
TPL IP3
TPL IP4
TPL IP5
TPL IP6
TP1IP1
TP1IP2
TP1IP3
TP1IP4
TP1IP5
TP1IP6
Increasing PENTAmer distances from the terminus
Linear recombinant PENTAmer library (type II). Only recombinant molecules with left terminal PENTAmer (TP L) are shown.

FIG. 15B

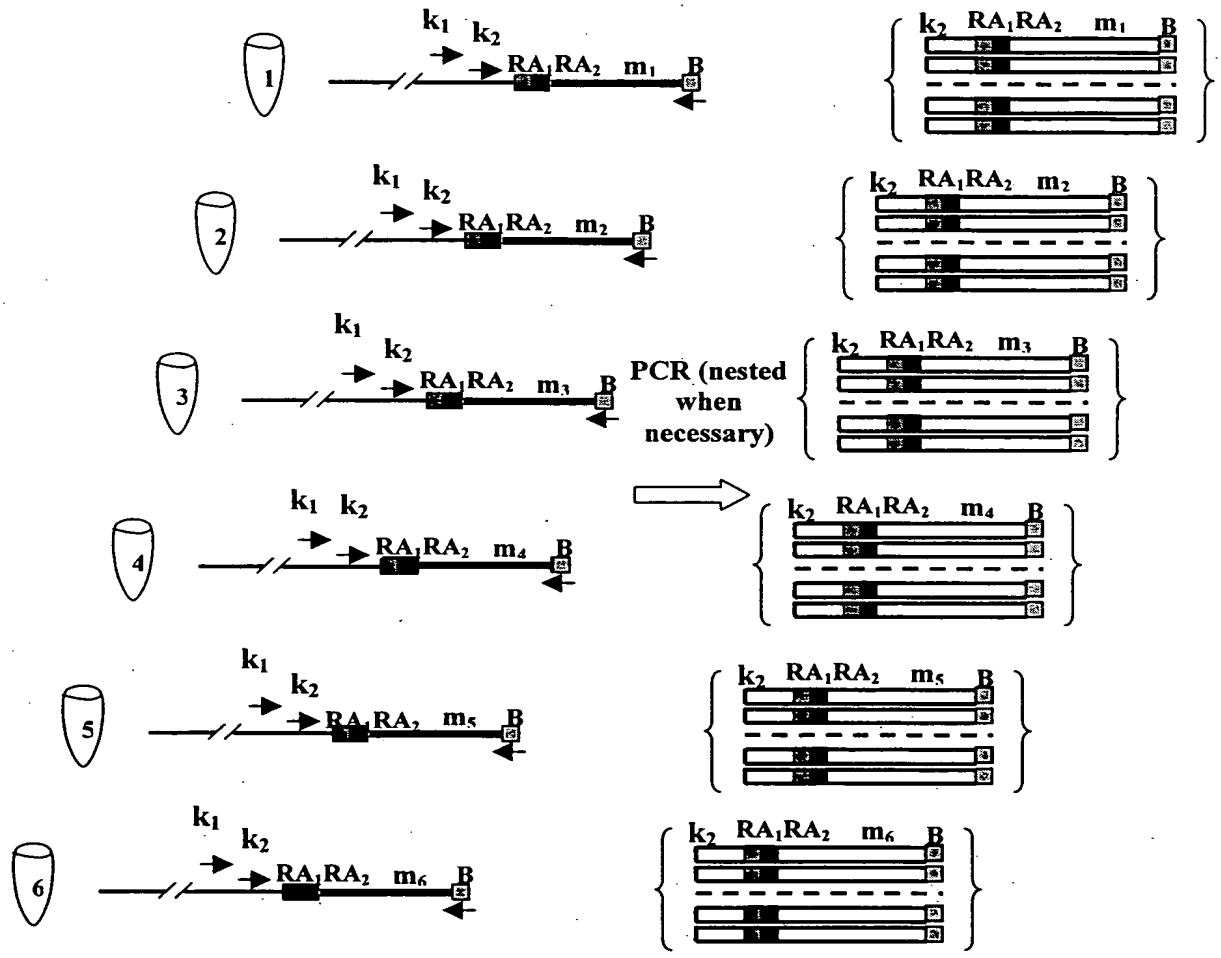
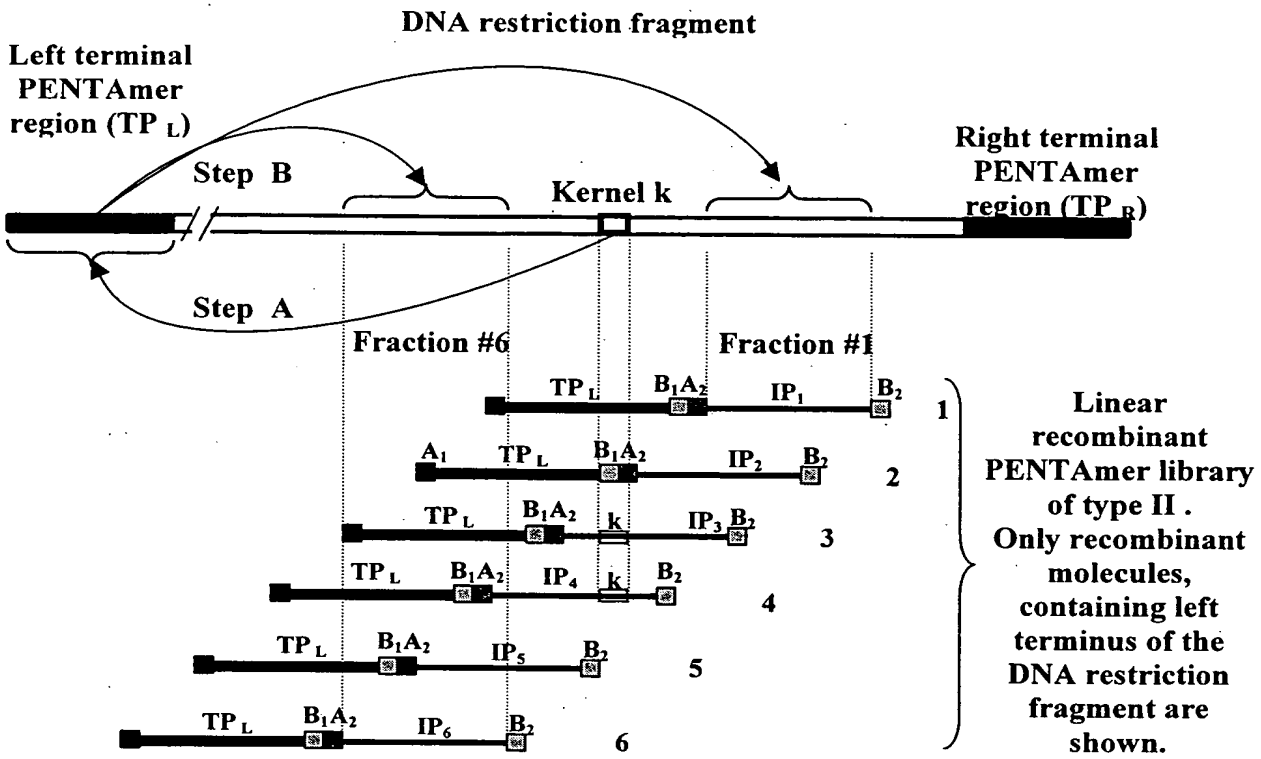

1
2
3
4
5
6
k1
k2
RA1RA2
m1
m2
m3
m4
m5
m6
B
PCR (nested when necessary)

FIG. 16



Step A. Amplification of terminal PENTAmers (TP) of a DNA restriction fragment using "One-tube" unordered library II, nested primers K_1 and K_2 from the internal "kernel" region k and adaptor-primer A_1 (only amplification of left terminal PENTAmer is shown)

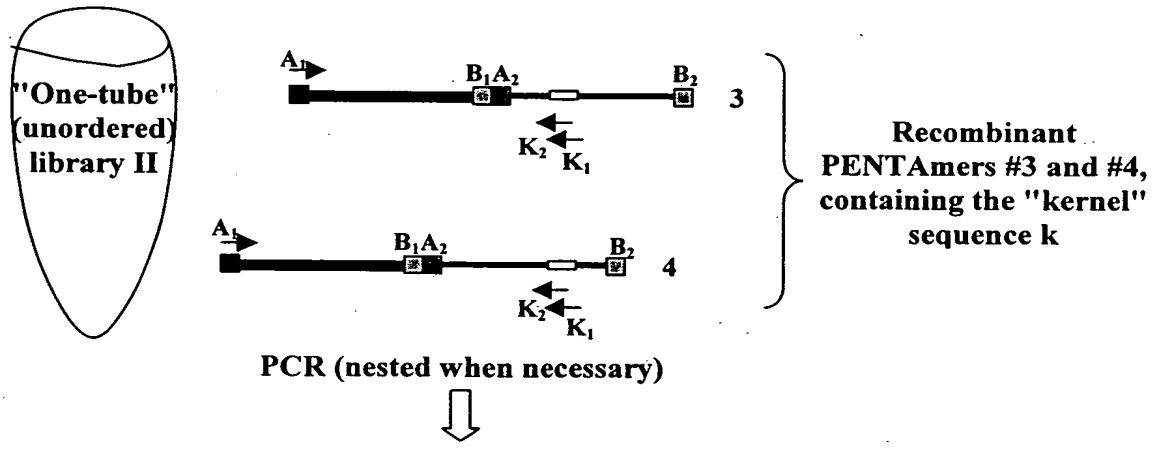
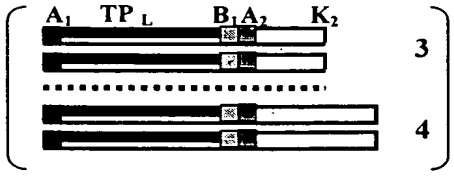


FIG. 17A



PCR products

Sequencing left terminal PENTAmer DNA (TP_L) using primers A_1 and/or B_1 followed by selection and construction of the PCR primers T_1 and T_2 from the terminal PENTAmer region

Step B. Amplification of the internal PENTAmer (IP) fractions of the DNA restriction fragment using linear ordered library II, nested primers T_1 and T_2 from the terminal PENTamere region TP and the adaptor-primer B_2

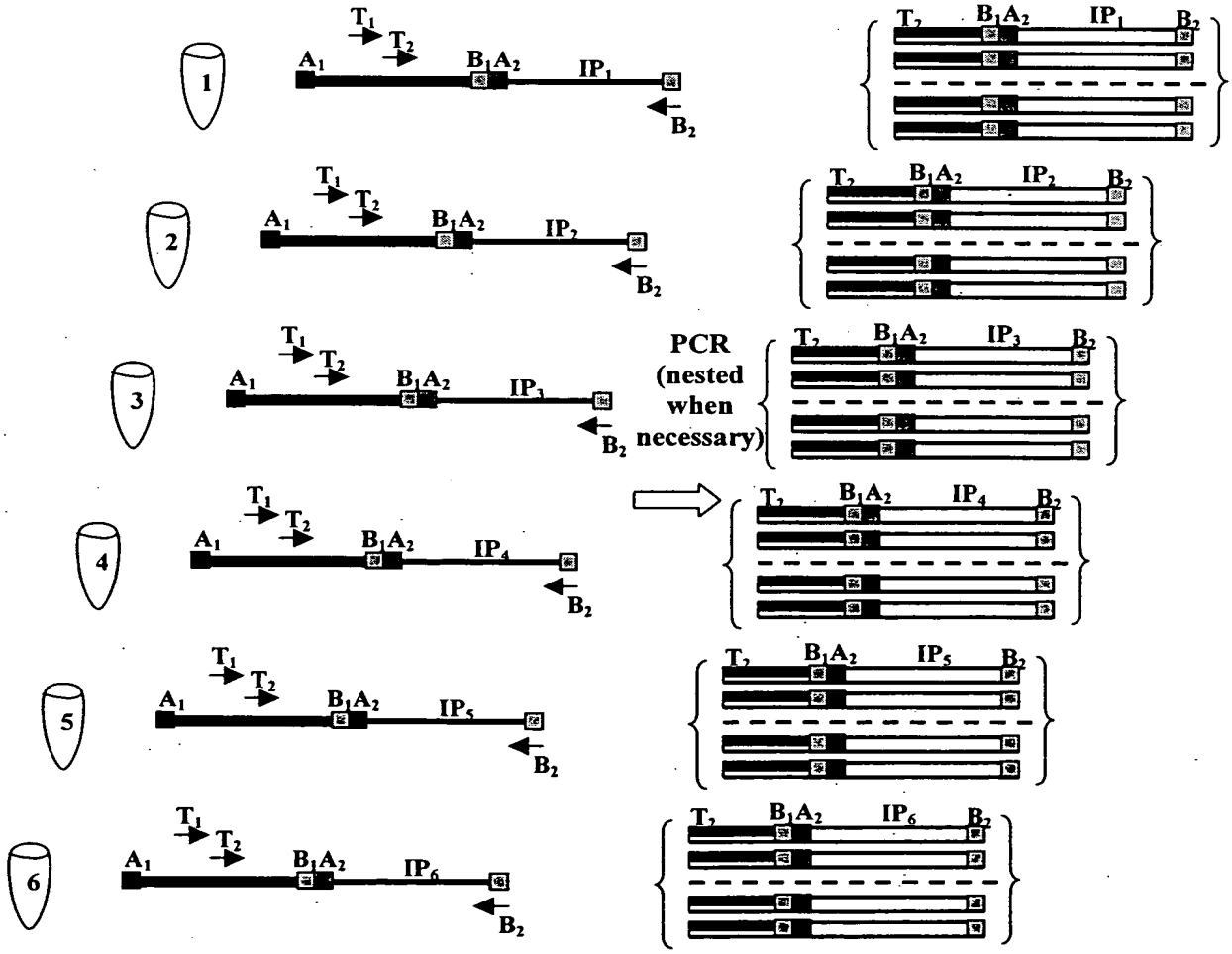
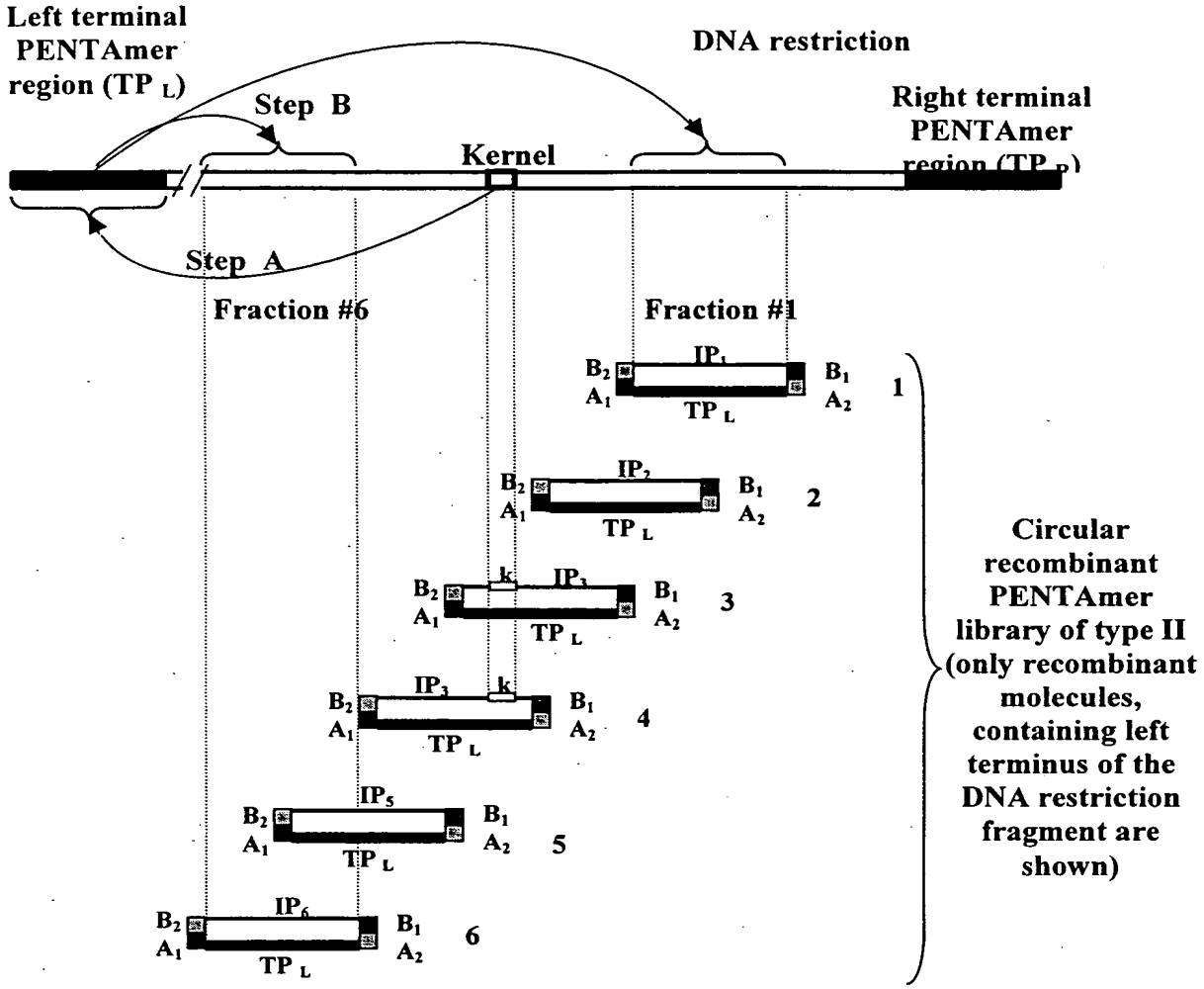
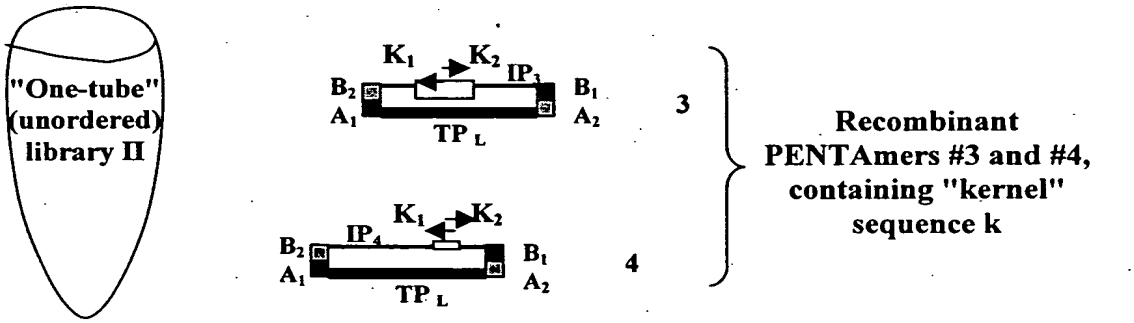


FIG. 17B



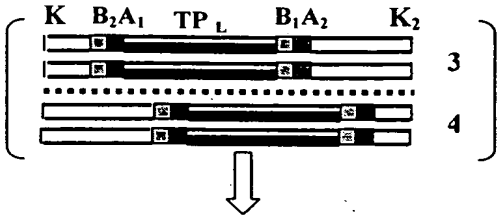
Step A. Amplification of terminal PENTAmers (TP) of a DNA restriction fragment using "One-tube" circular library II, inverse primers K_1 and K_2 from the internal "kernel" region k



Inverse PCR (nested when necessary)

⇩

FIG. 17C



Sequencing a terminal PENTAmer DNA (TP) using primers A_1 and /or B_1 followed by selection and construction of two inverse PCR primers T_1 and T_2

Step B. Amplification of the internal PENTAmer (IP) fractions of the DNA restriction fragment using inverse primers T_1 and T_2 from the terminal PENTAmer region and ordered circular library II

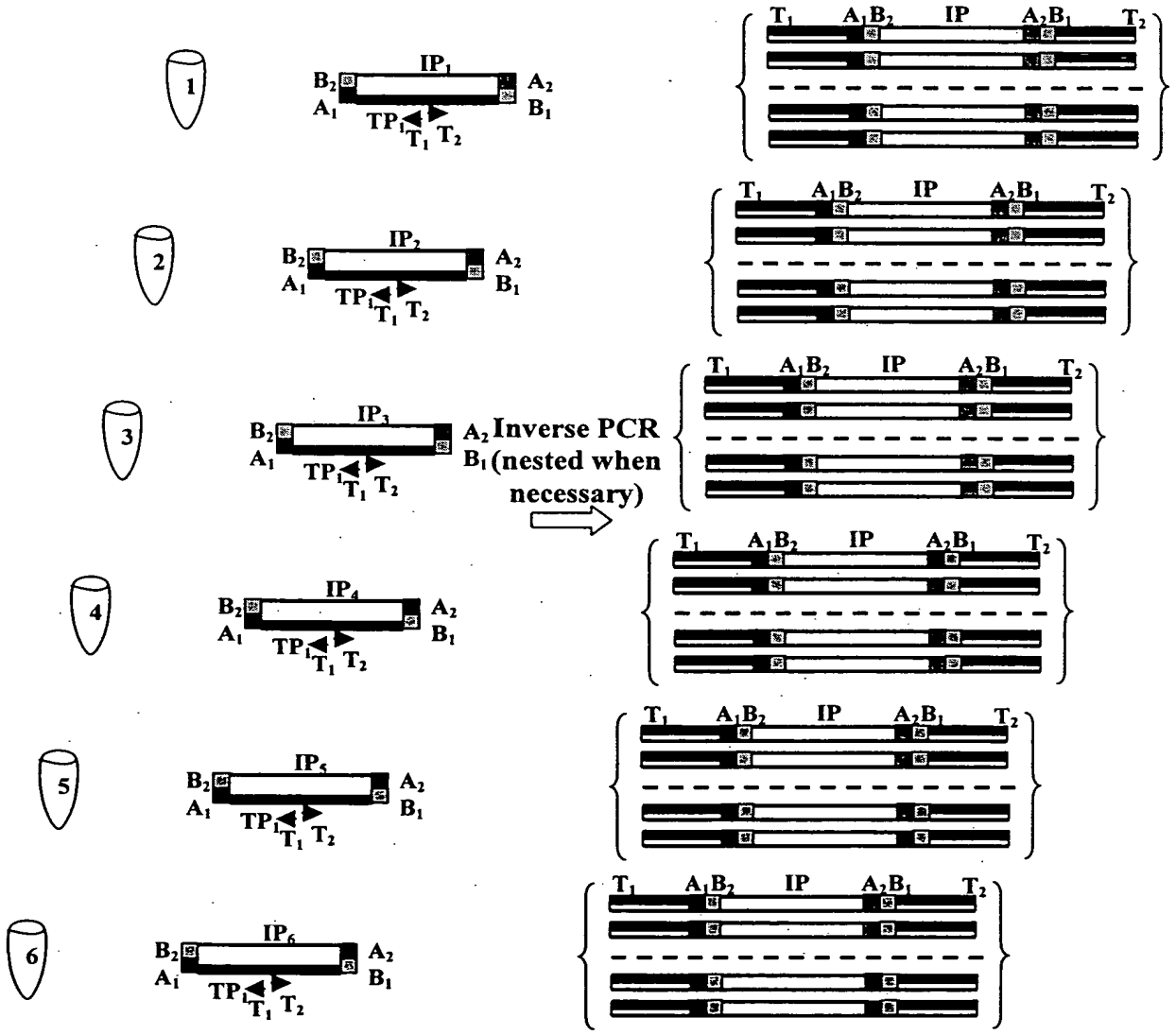


FIG. 17D

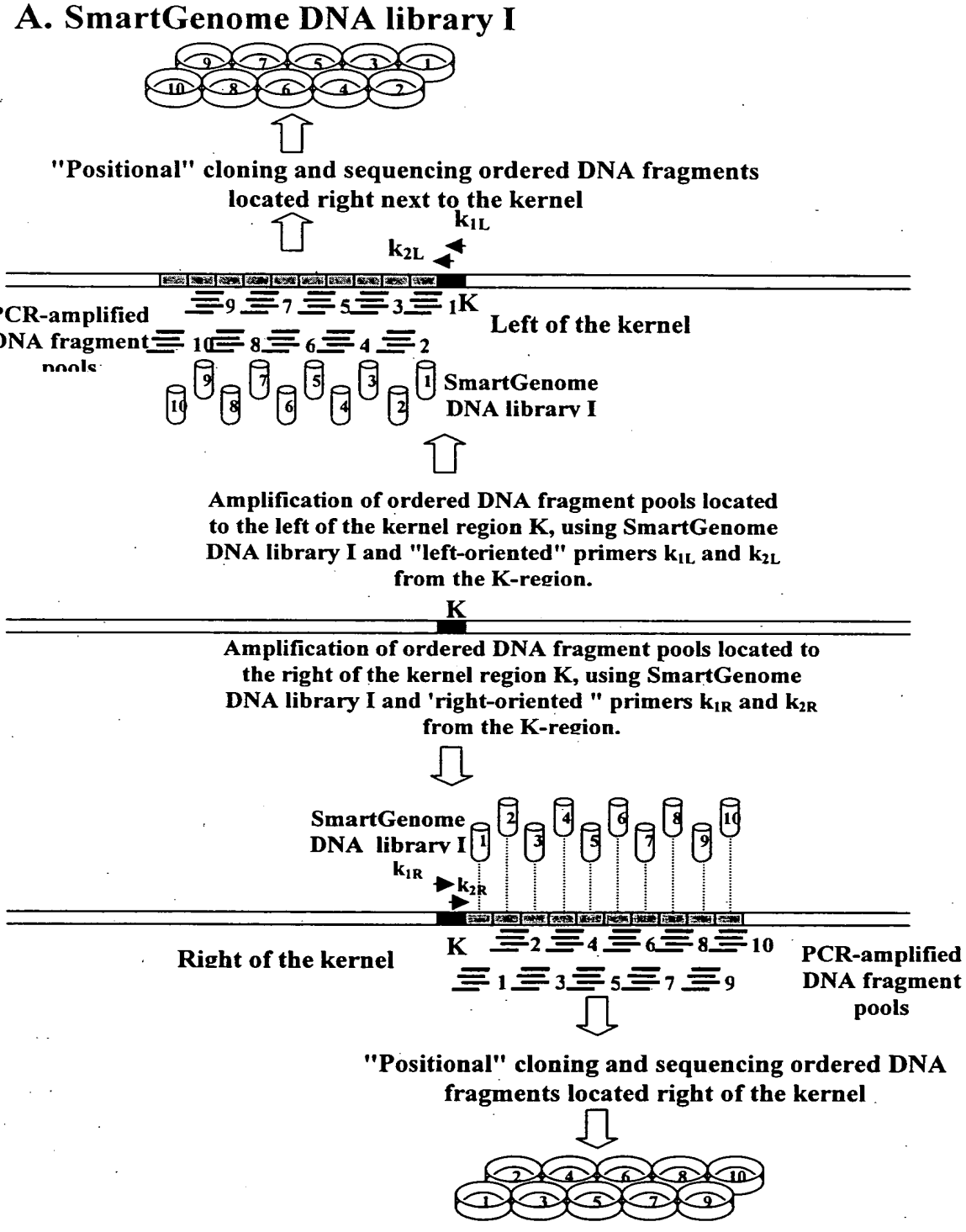
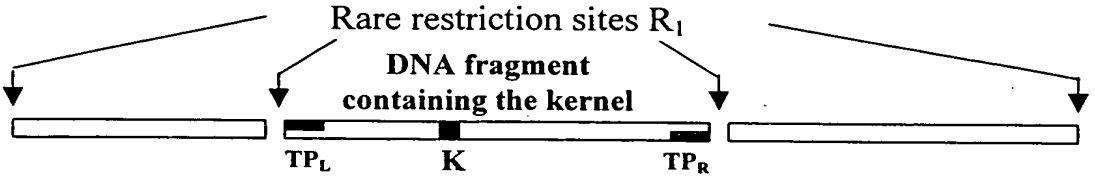
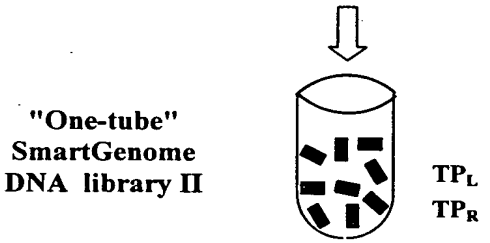

A. SmartGenome DNA library I
"Positional" cloning and sequencing ordered DNA fragments located right next to the kernel
k1L
k2L
PCR-amplified DNA fragment pools
Left of the kernel
SmartGenome DNA library I
Amplification of ordered DNA fragment pools located to the left of the kernel region K, using SmartGenome DNA library I and "left-oriented" primers k1L and k2L from the K-region.
K
Amplification of ordered DNA fragment pools located to the right of the kernel region K, using SmartGenome DNA library I and 'right-oriented " primers k1R and k2R from the K-region.
SmartGenome DNA library I
k1R
k2R
Right of the kernel
PCR-amplified DNA fragment pools
"Positional" cloning and sequencing ordered DNA fragments located right of the kernel

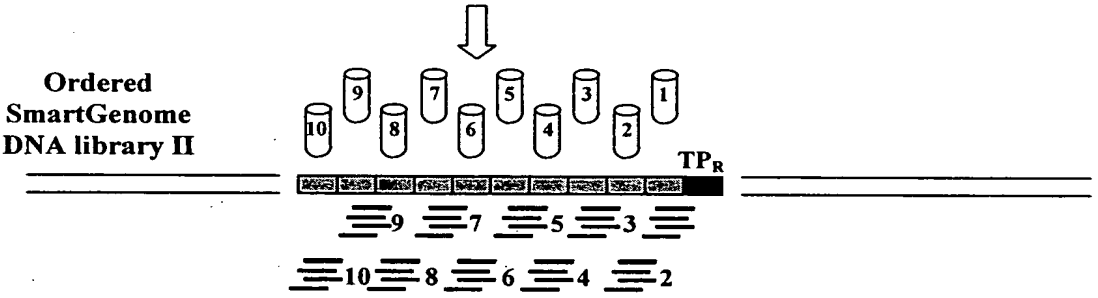
FIG. 18A



Amplification, isolation and sequencing of termini TP_L and TP_R of the DNA restriction fragment R with the kernel sequence using unordered "One-tube" SmartGenome DNA library II and primers k_1 and k_2 from the K-region



Amplification of the ordered internal DNA molecules located within the same DNA restriction fragment R using ordered SmartGenome DNA library II and primers from the terminal regions



"Positional" cloning and sequencing ordered DNA molecules within the restriction fragment containing the kernel

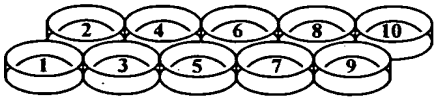
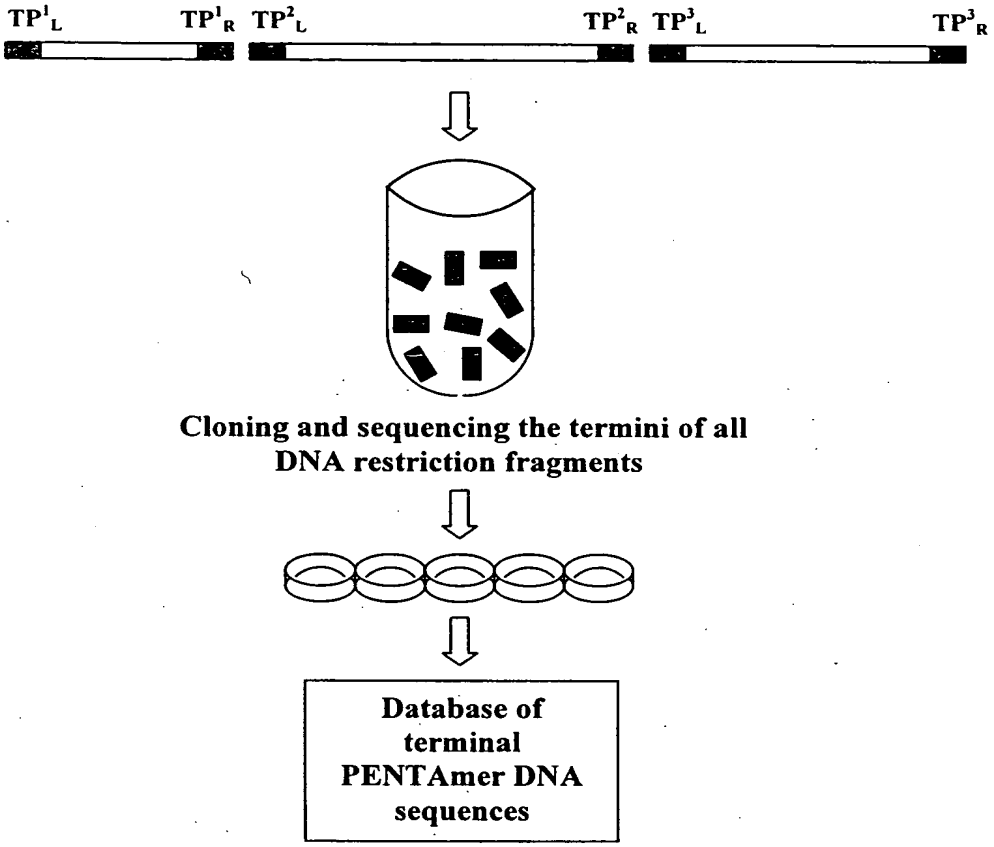


FIG. 18B

Step 1 Linear amplification of termini (TP_L and TP_R) of all DNA restriction fragments using unordered "One-tube" SmartGenome DNA library II and adaptor-primers A_1 and B_1 (see Fig. 17A)



Step 2 Amplification of the ordered internal DNA fractions located within the DNA restriction fragments using ordered SmartGenome DNA library II and primers from the terminal regions

Step 3 Amplification, isolation and sequencing of the "linking" DNA fractions using ordered SmartGenome DNA library I and primers within the adjacent terminus region

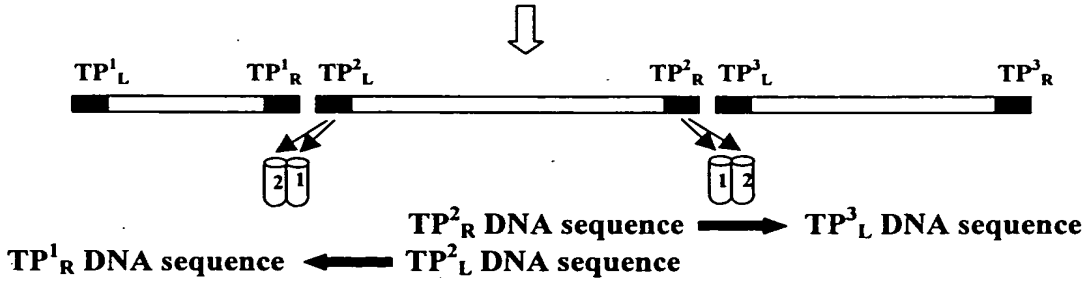


FIG. 18C

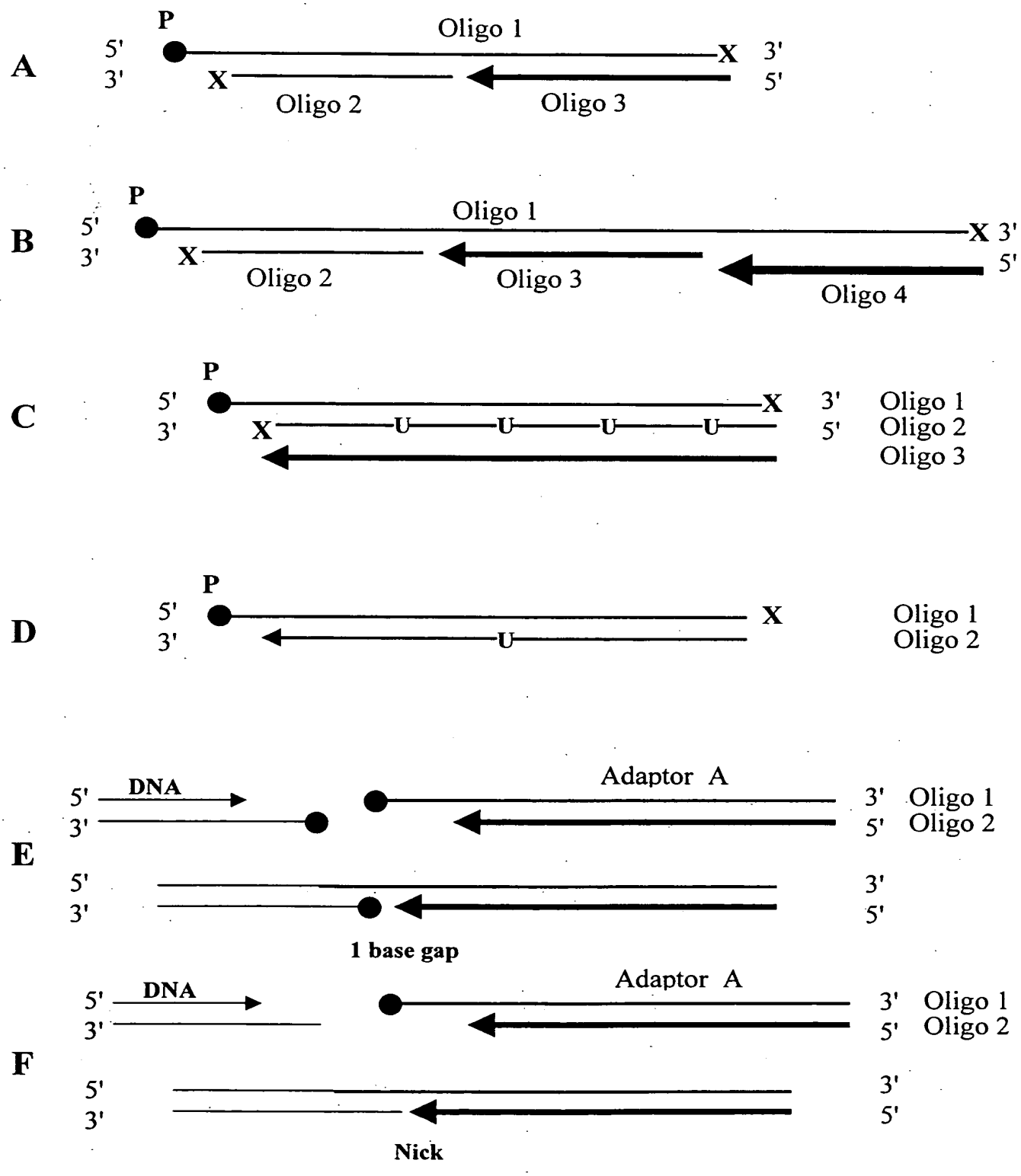

A
P
Oligo 1
5'
3'
X
3'
5'
X
Oligo 2
Oligo 3
B
P
Oligo 1
5'
3'
X
X 3'
5'
Oligo 2
Oligo 3
Oligo 4
C
P
5'
3'
X
3'
5'
X
U
U
U
U
Oligo 1
Oligo 2
Oligo 3
D
P
5'
3'
X
U
Oligo 1
Oligo 2
E
5'
DNA
3'
Adaptor A
3'
Oligo 1
5'
Oligo 2
5'
3'
3'
5'
1 base gap
F
5'
DNA
3'
Adaptor A
3'
Oligo 1
5'
Oligo 2
5'
3'
3'
5'
Nick

FIG. 19

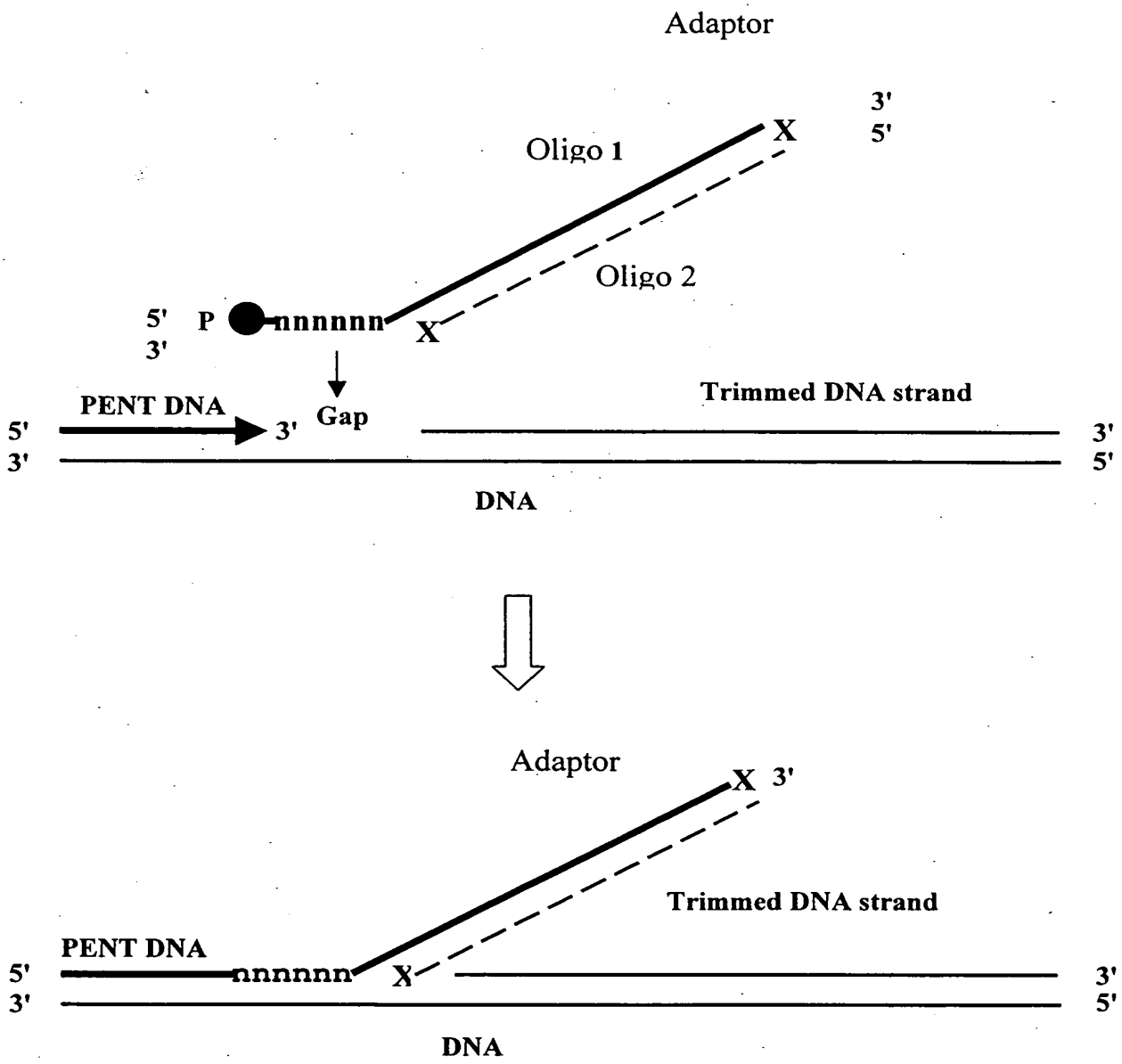

Adaptor
3'
5'
X
Oligo 1
Oligo 2
5'
P
nnnnnn
X
3'
PENT DNA
Gap
Trimmed DNA strand
5'
3'
3'
5'
DNA
Adaptor
X
3'
Trimmed DNA strand
PENT DNA
5'
nnnnnn
X
3'
3'
5'
DNA

FIG. 20

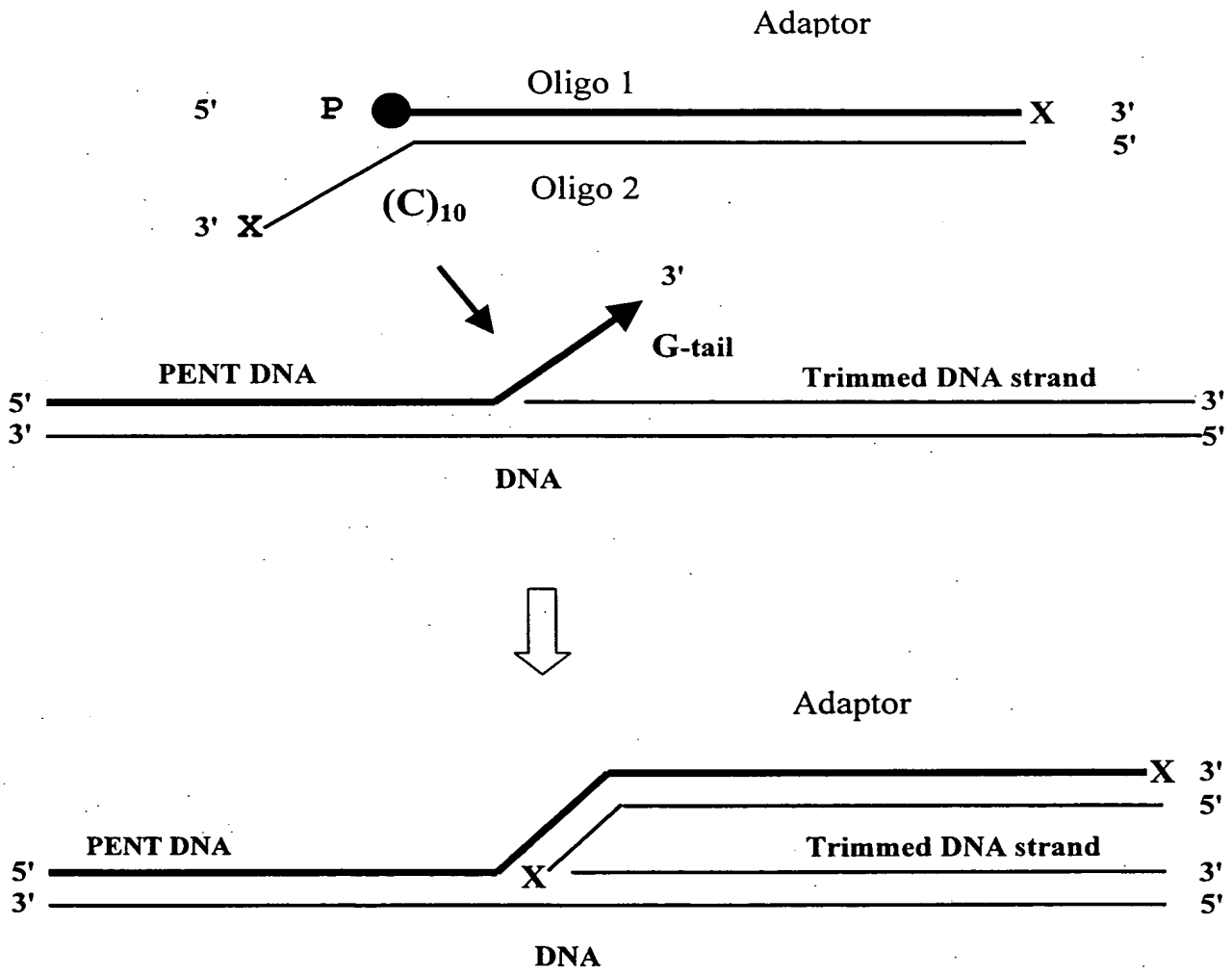

Adaptor
Oligo 1
5'
P
X
3'
5'
Oligo 2
3'
X
(C)10
3'
G-tail
PENT DNA
Trimmed DNA strand
5'
3'
3'
5'
DNA
Adaptor
X
3'
5'
PENT DNA
Trimmed DNA strand
5'
X
3'
3'
5'
DNA

FIG.21

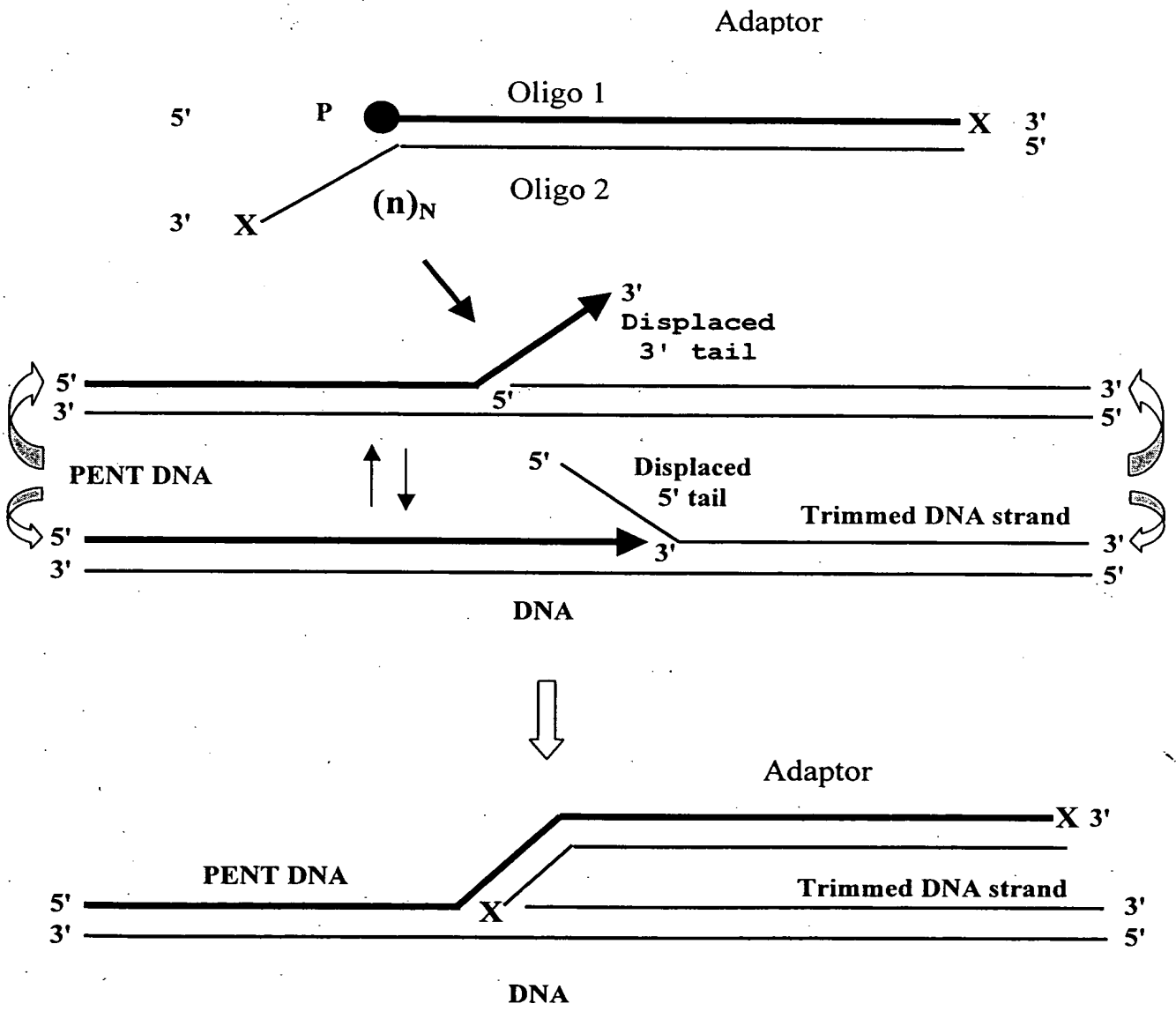

Adaptor
Oligo 1
5'
P
X
3'
5'
3'
X
$(n)_N$
Oligo 2
3'
Displaced
3' tail
5'
3'
5'
3'
PENT DNA
5'
Displaced
5' tail
Trimmed DNA strand
5'
3'
3'
5'
DNA
Adaptor
X 3'
PENT DNA
Trimmed DNA strand
5'
X
3'
3'
5'
DNA

FIG. 22

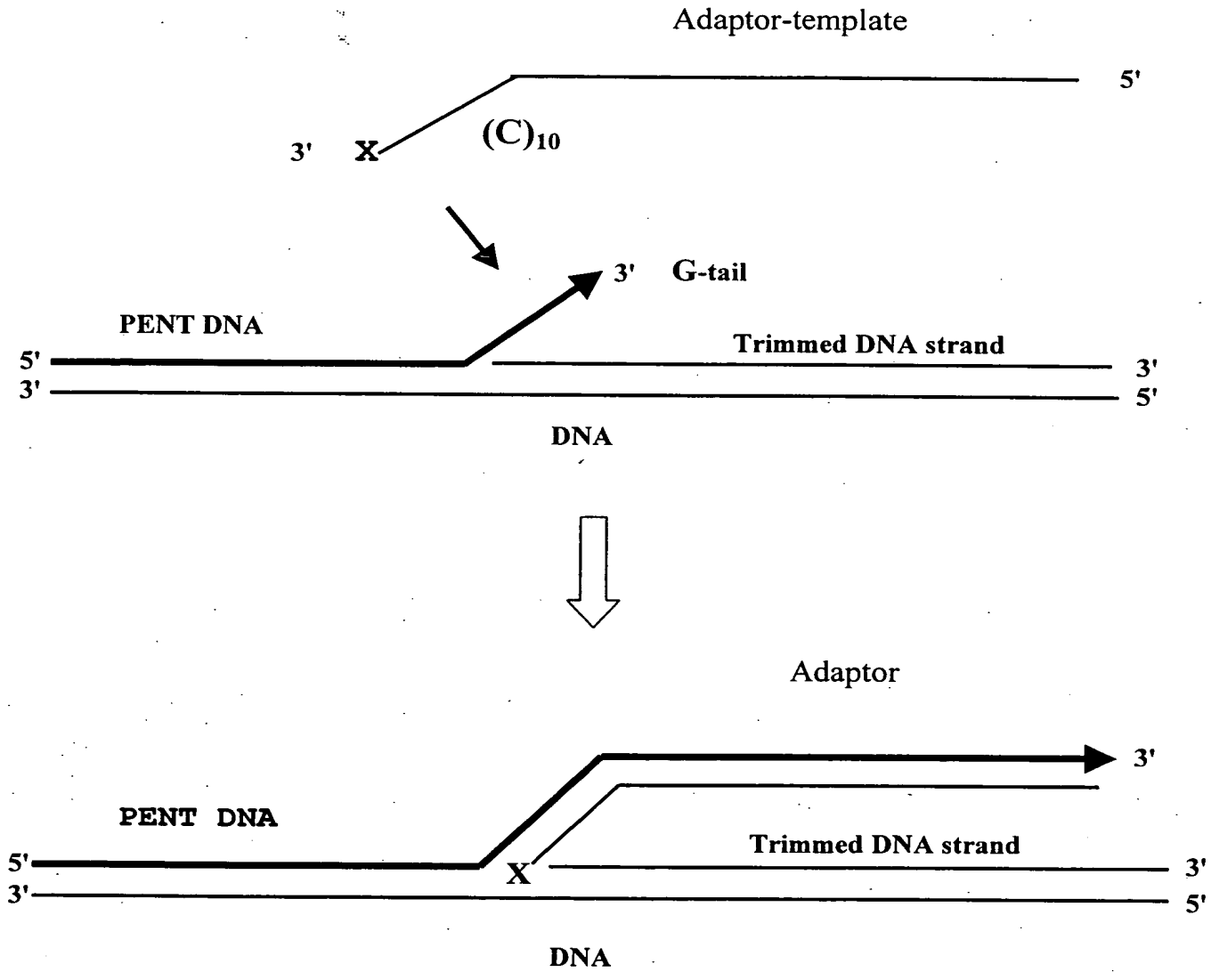

Adaptor-template
5'
3'
X
(C)10
3'
G-tail
PENT DNA
Trimmed DNA strand
5'
3'
3'
5'
DNA
Adaptor
3'
PENT DNA
Trimmed DNA strand
5'
X
3'
3'
5'
DNA

FIG. 23

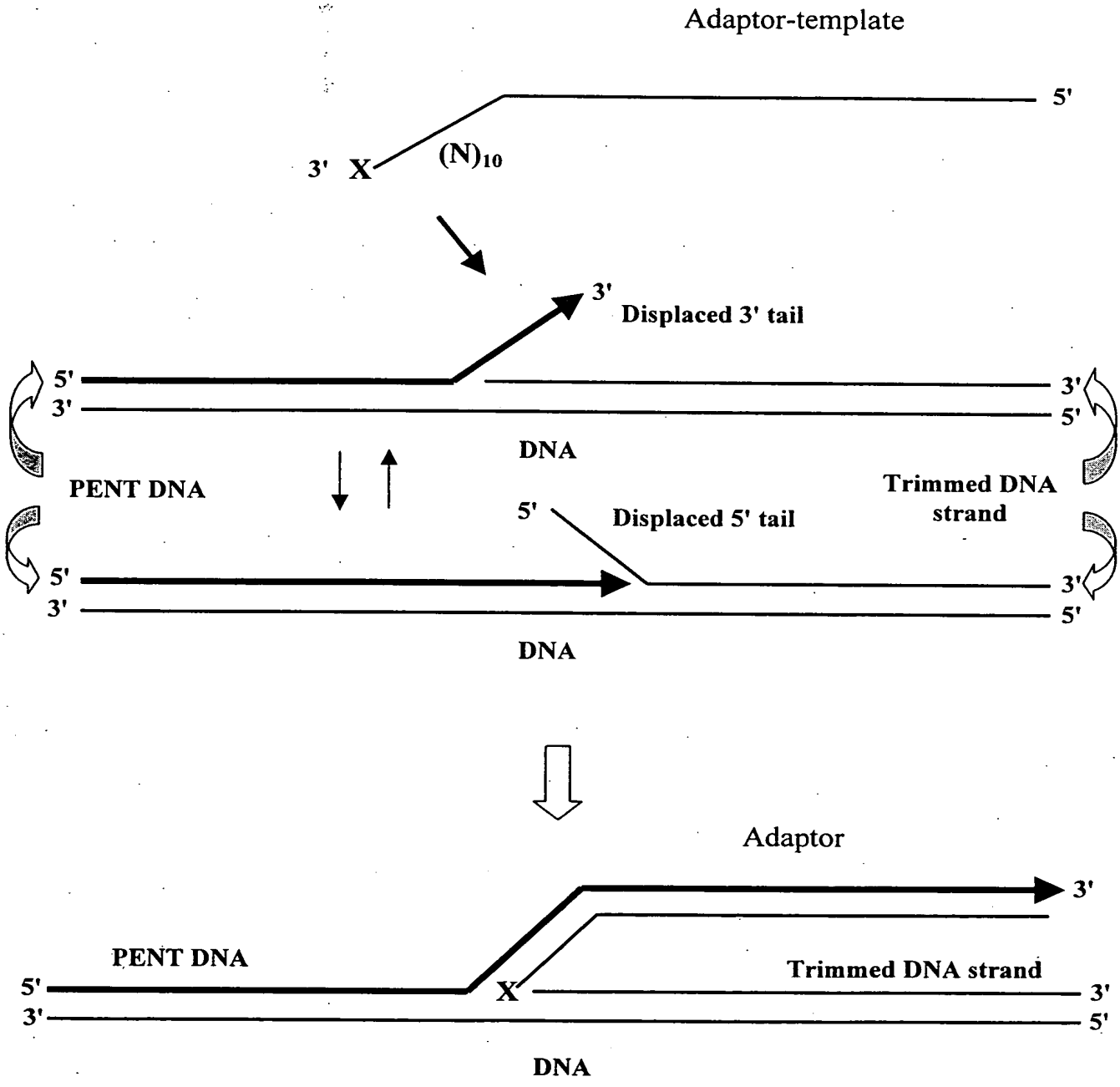

Adaptor-template
5'
3'
X
(N)10
3'
Displaced 3' tail
5'
3'
3'
5'
DNA
PENT DNA
Trimmed DNA strand
5'
Displaced 5' tail
5'
3'
3'
5'
DNA
Adaptor
3'
PENT DNA
Trimmed DNA strand
5'
X
3'
3'
5'
DNA

FIG. 24

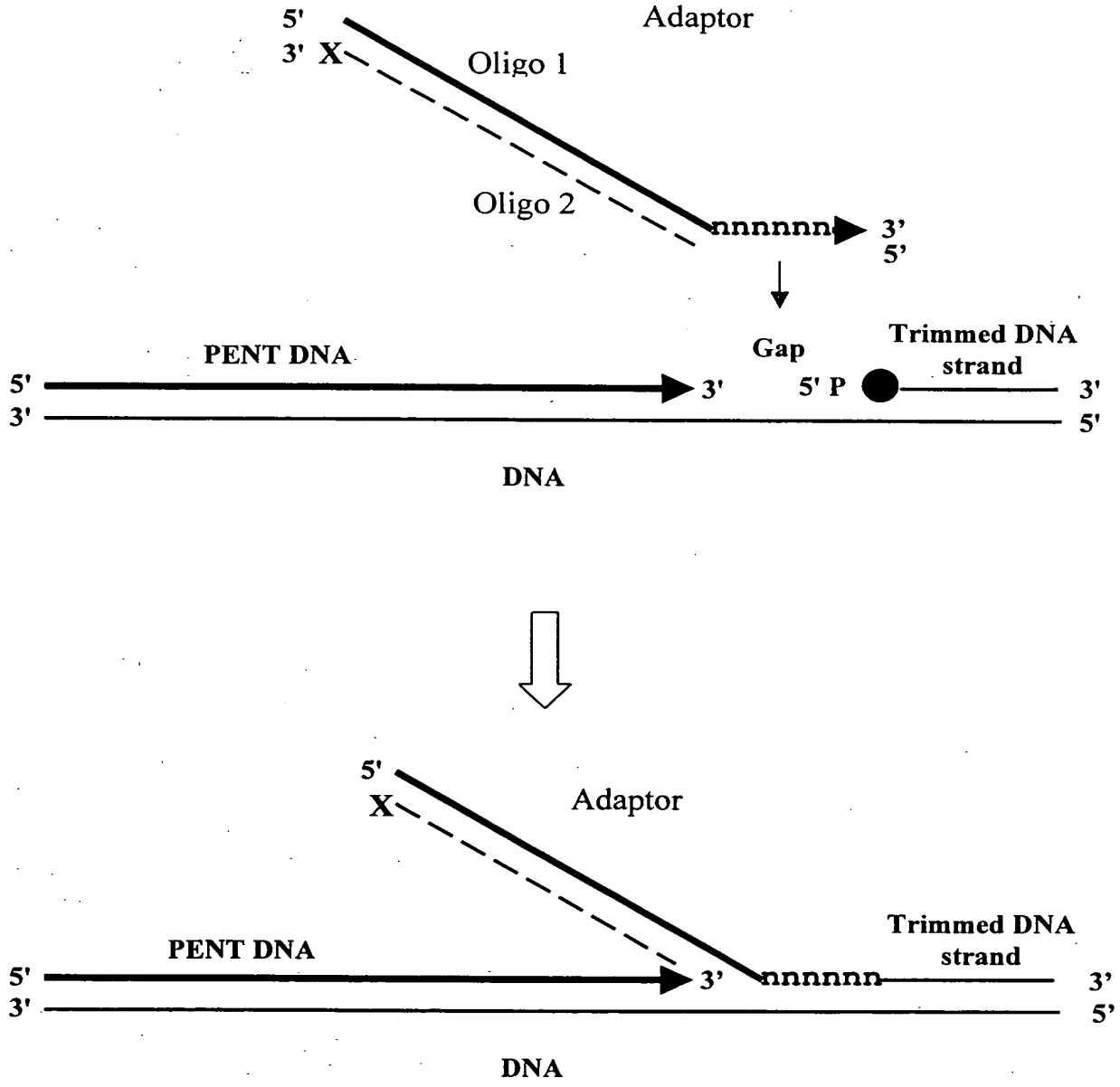

5'
3' X
Adaptor
Oligo 1
Oligo 2
nnnnnn
3'
5'
PENT DNA
Gap
Trimmed DNA strand
5'
3'
5' P
3'
3'
5'
DNA
5'
X
Adaptor
PENT DNA
Trimmed DNA strand
5'
3'
3'
nnnnnn
3'
5'
DNA

FIG. 25

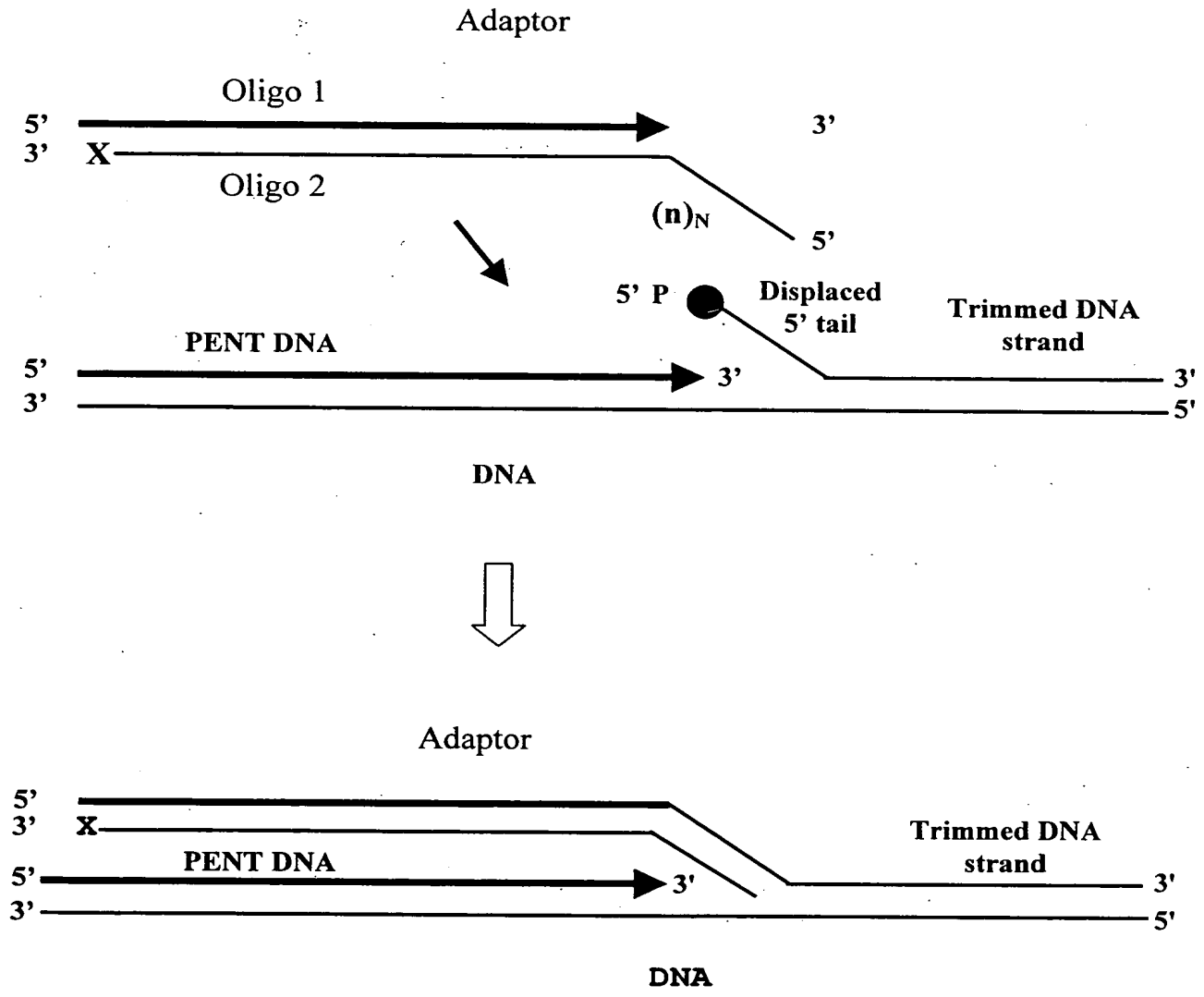

Adaptor
Oligo 1
5'
3'
X
3'
Oligo 2
$(n)_N$
5'
5' P
Displaced
5' tail
Trimmed DNA
strand
PENT DNA
5'
3'
3'
3'
5'
DNA
Adaptor
5'
3'
X
Trimmed DNA
strand
PENT DNA
5'
3'
3'
3'
5'
DNA

FIG. 26

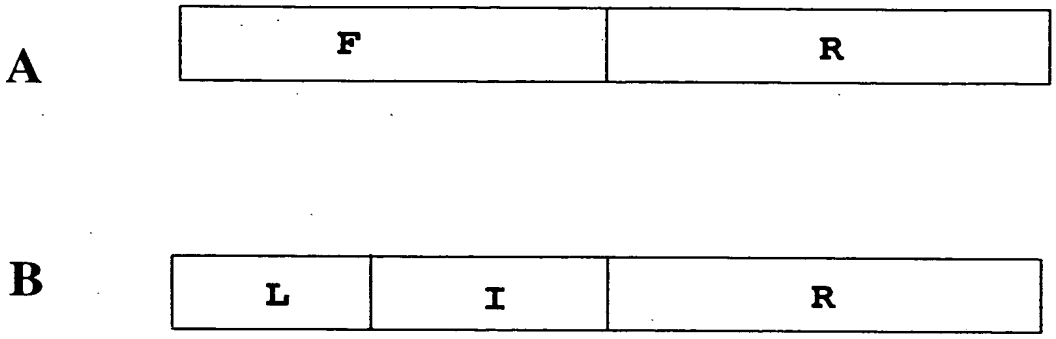
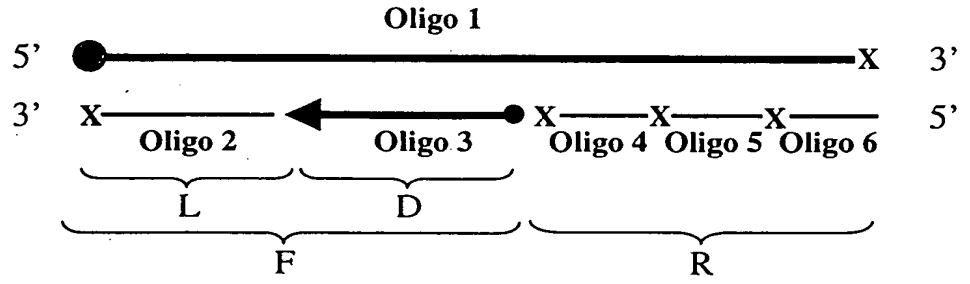
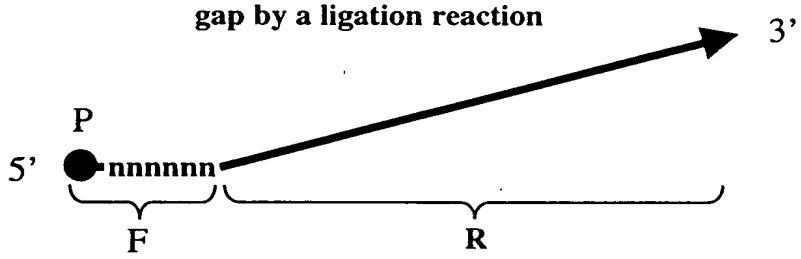


FIG. 27

A Up-stream terminus-attaching nick-translation recombination adaptor RA



B Down-stream nick-attaching recombination adaptor RB-3' (I) targeted to a gap by a ligation reaction



C Down-stream nick-attaching recombination adaptor RB-3' (II) targeted to a poly-G tail by a ligation reaction

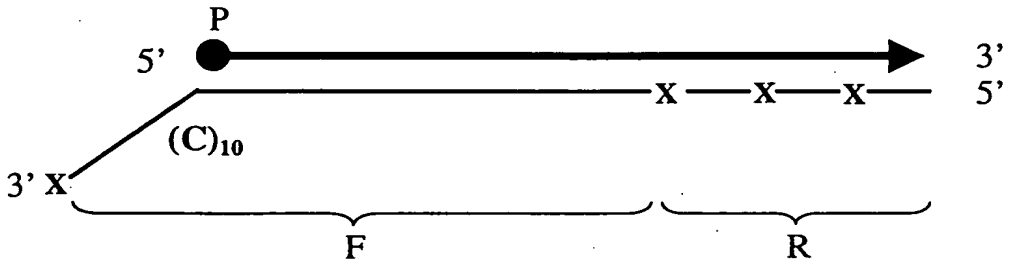
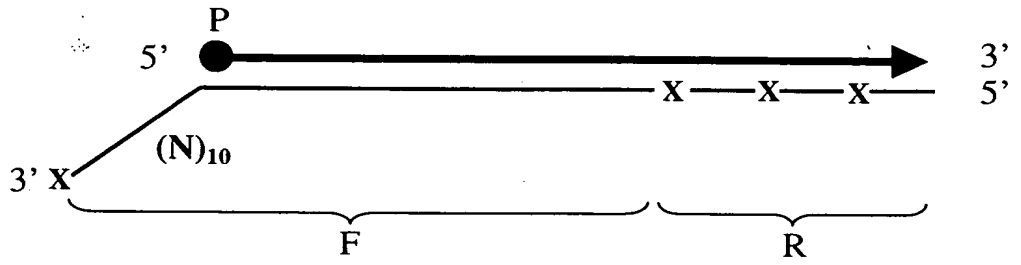
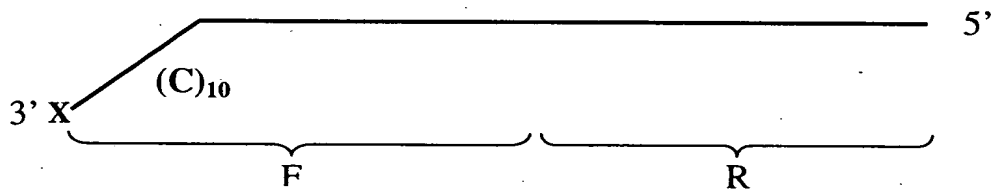


FIG. 28A

D **Down-stream nick-attaching recombination adaptor RB-3' (III) targeted to a displaced 3' DNA tail by a ligation reaction**



E **Down-stream nick-attaching recombination adaptor RB-3' (IV) targeted to a poly-G tail as a template for a polymerization-extension reaction**



F **Down-stream nick-attaching recombination adaptor RB-3' (V) targeted to a displaced 3' DNA tail as a template for a polymerization-extension reaction**

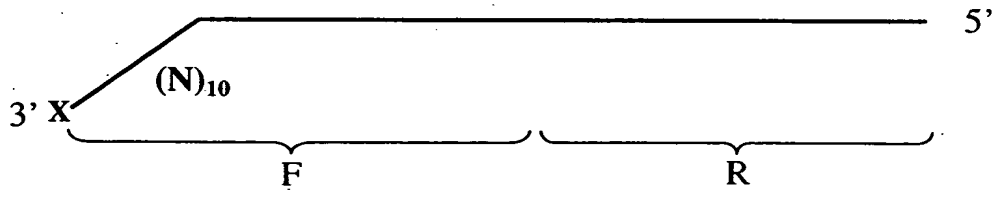
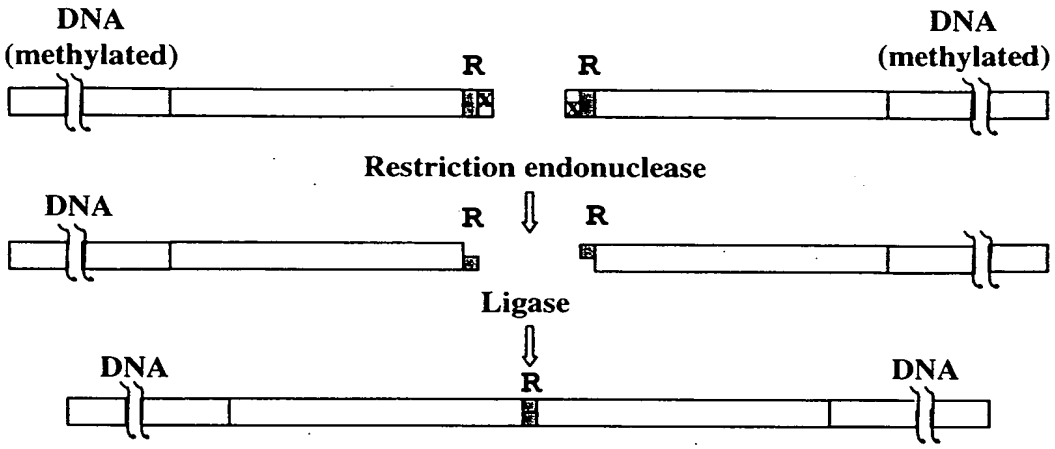


FIG. 28B

A RecAdaptors-Class I



B RecAdaptors-Class II

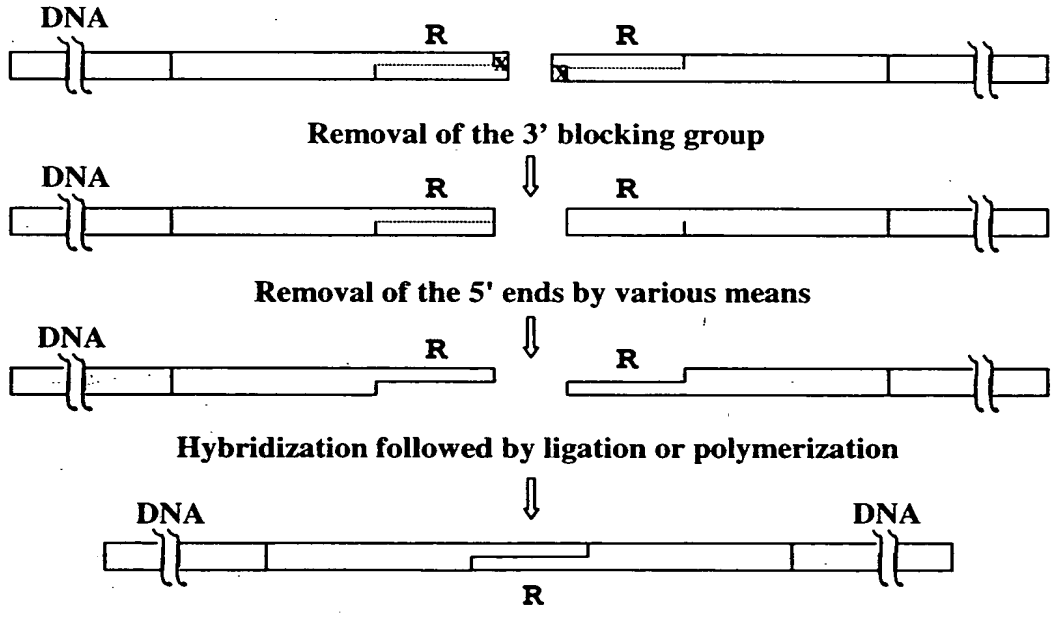


FIG. 29

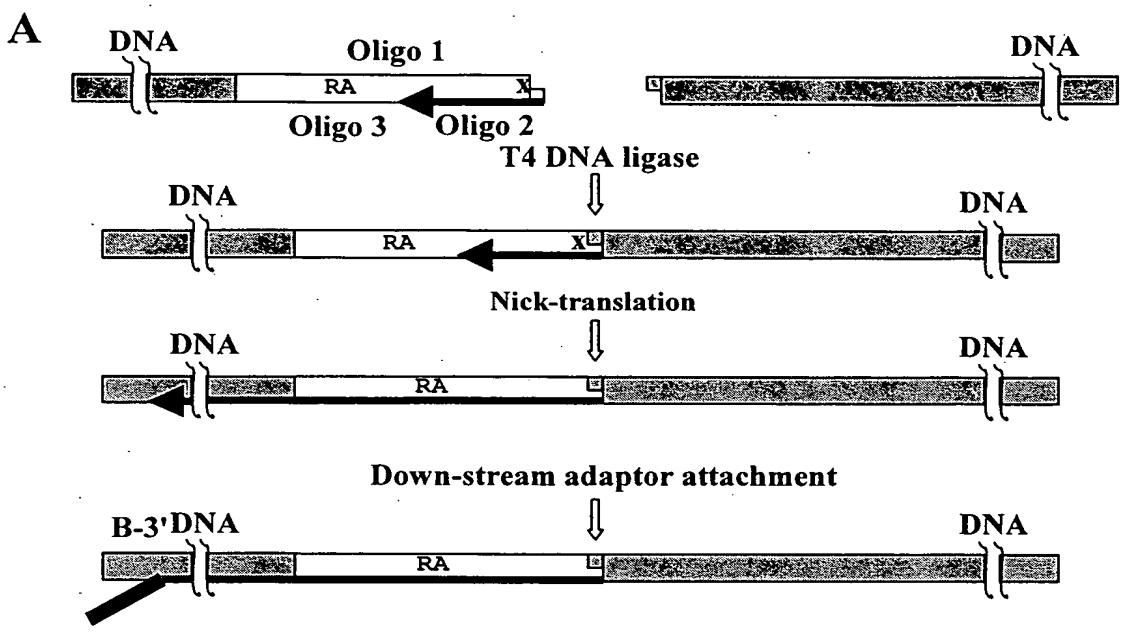

A
DNA
Oligo 1
RA
X
DNA
Oligo 3
Oligo 2
T4 DNA ligase
DNA
DNA
RA
X
Nick-translation
DNA
DNA
RA
Down-stream adaptor attachment
B-3'DNA
DNA
RA

FIG. 30A

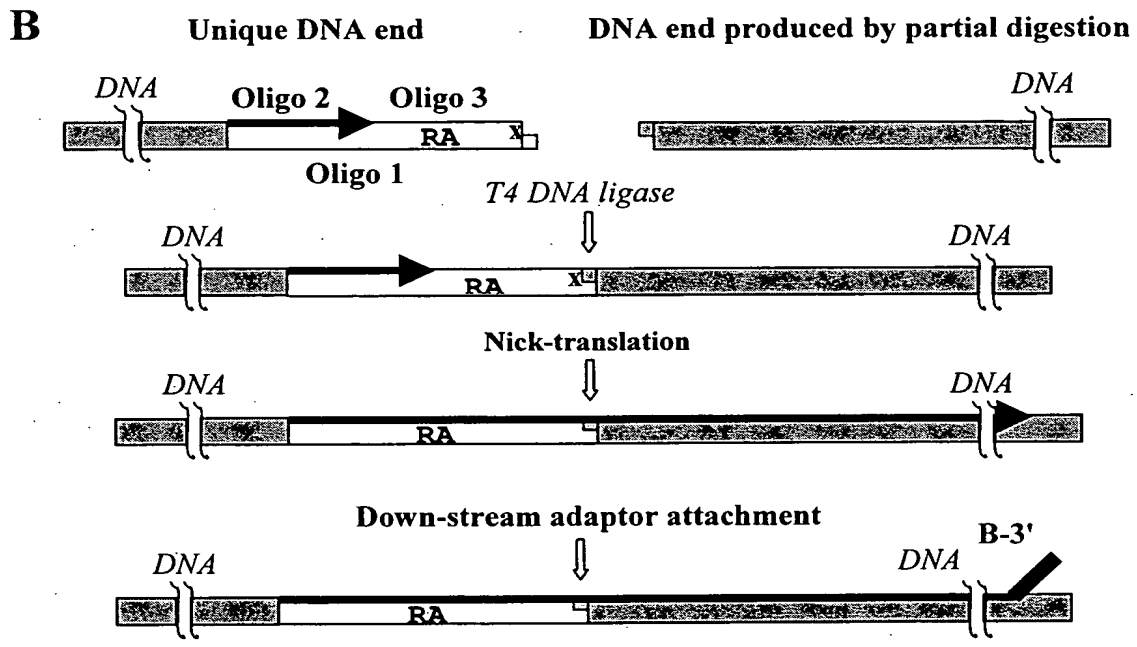

B
Unique DNA end
DNA end produced by partial digestion
DNA
Oligo 2
Oligo 3
RA
X
Oligo 1
DNA
T4 DNA ligase
DNA
RA
X
DNA
Nick-translation
DNA
RA
DNA
Down-stream adaptor attachment
B-3'
DNA
RA
DNA

FIG. 30B

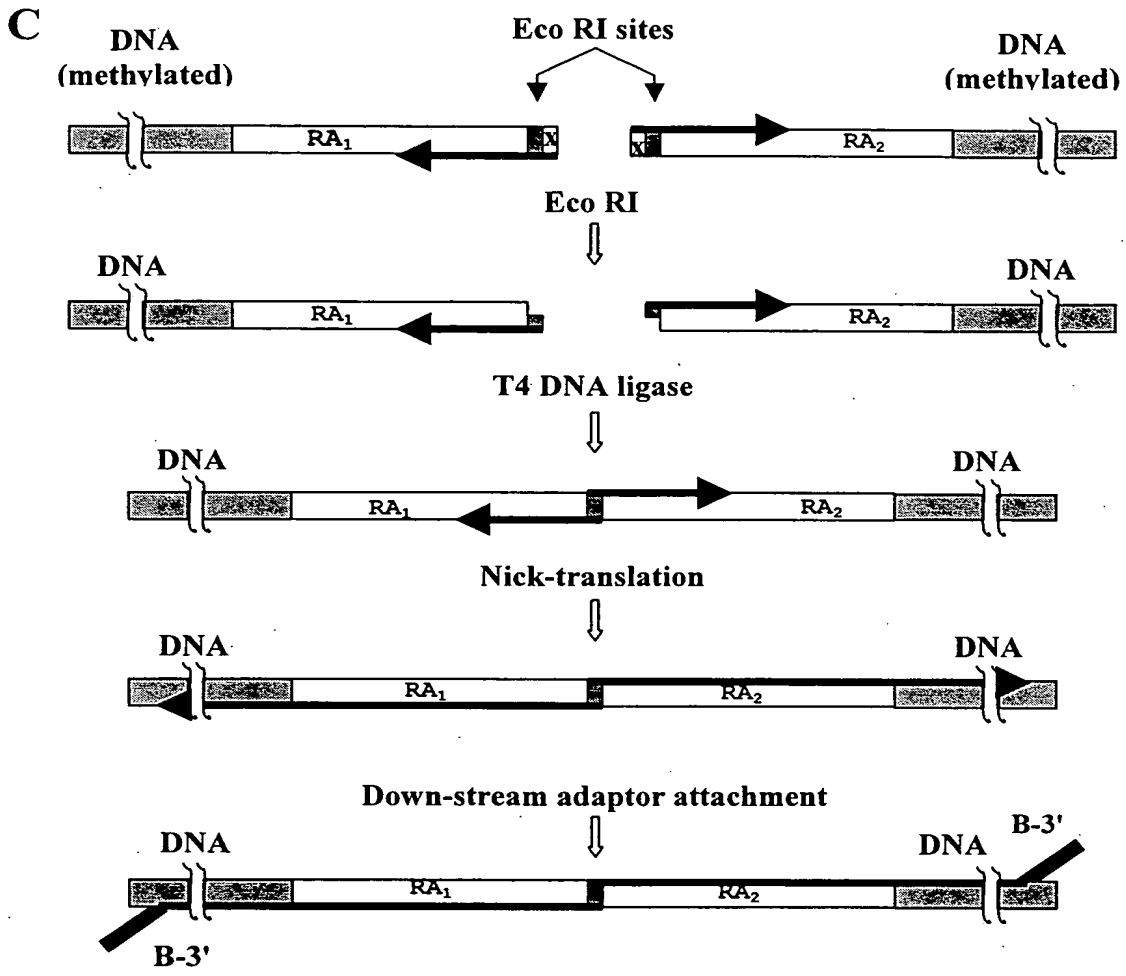

C
DNA
(methylated)
Eco RI sites
DNA
(methylated)
RA1
RA2
Eco RI
DNA
DNA
RA1
RA2
T4 DNA ligase
DNA
DNA
RA1
RA2
Nick-translation
DNA
DNA
RA1
RA2
Down-stream adaptor attachment
DNA
DNA
B-3'
RA1
RA2
B-3'

FIG. 30C

D

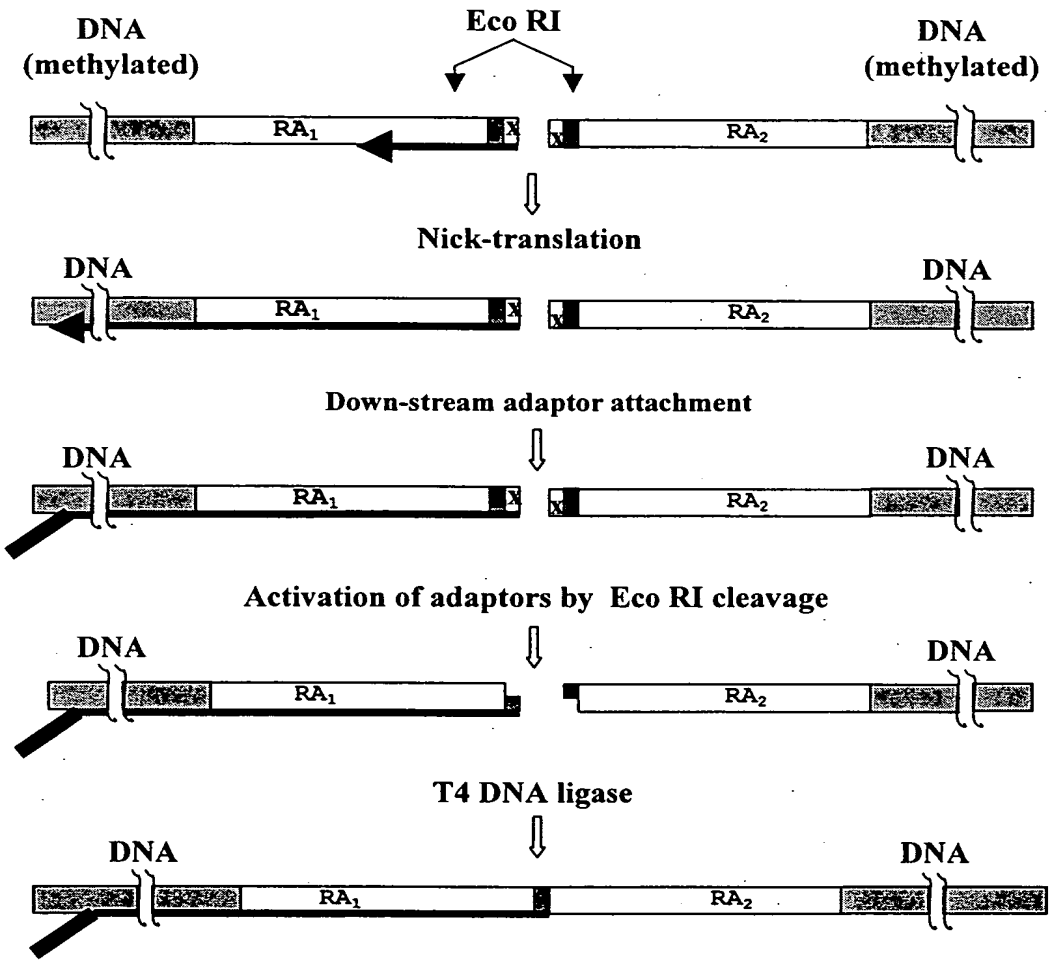

DNA
(methylated)
Eco RI
DNA
(methylated)
RA1
RA2
Nick-translation
DNA
DNA
RA1
RA2
Down-stream adaptor attachment
DNA
DNA
RA1
RA2
Activation of adaptors by Eco RI cleavage
DNA
DNA
RA1
RA2
T4 DNA ligase
DNA
DNA
RA1
RA2

FIG. 30D

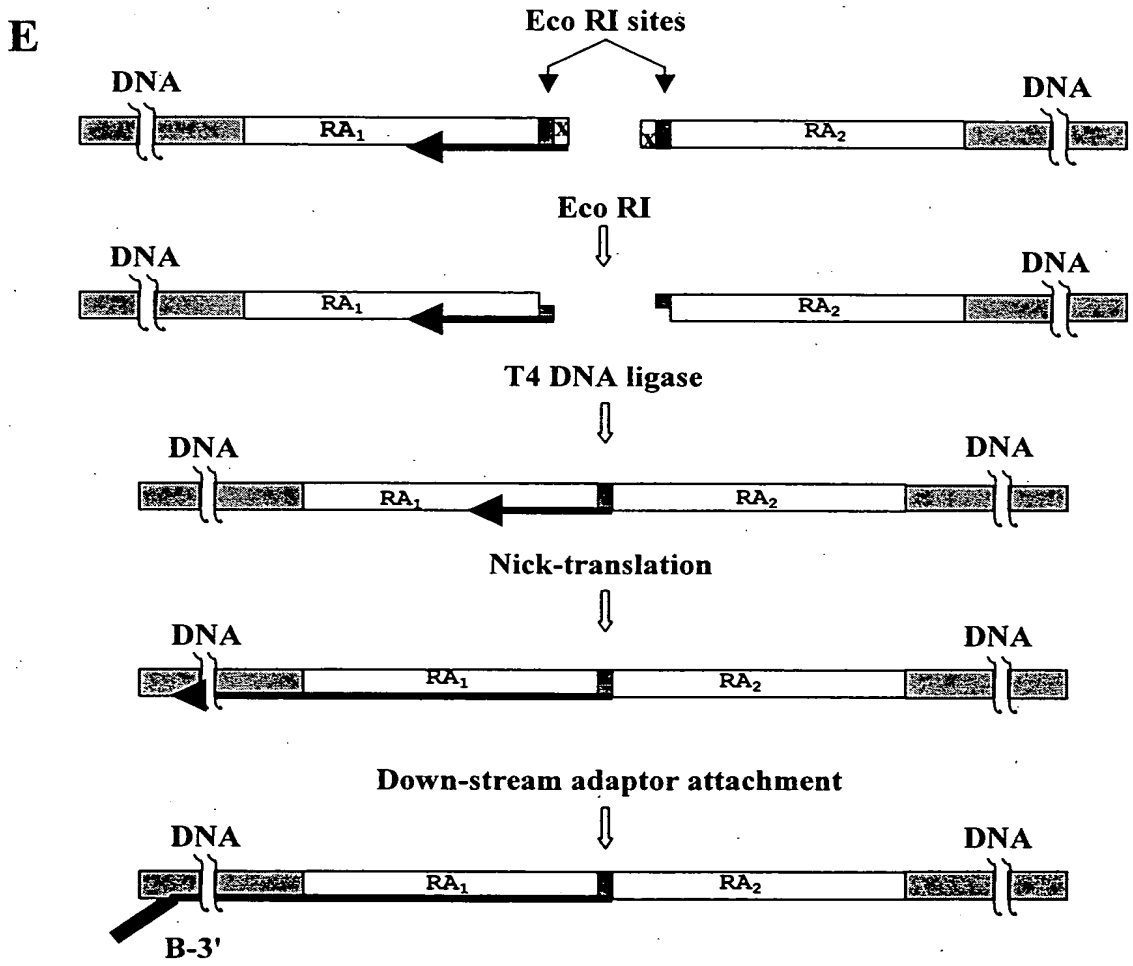

E
Eco RI sites
DNA
DNA
RA1
RA2
Eco RI
DNA
DNA
RA1
RA2
T4 DNA ligase
DNA
DNA
RA1
RA2
Nick-translation
DNA
DNA
RA1
RA2
Down-stream adaptor attachment
DNA
DNA
RA1
RA2
B-3'

FIG. 30E

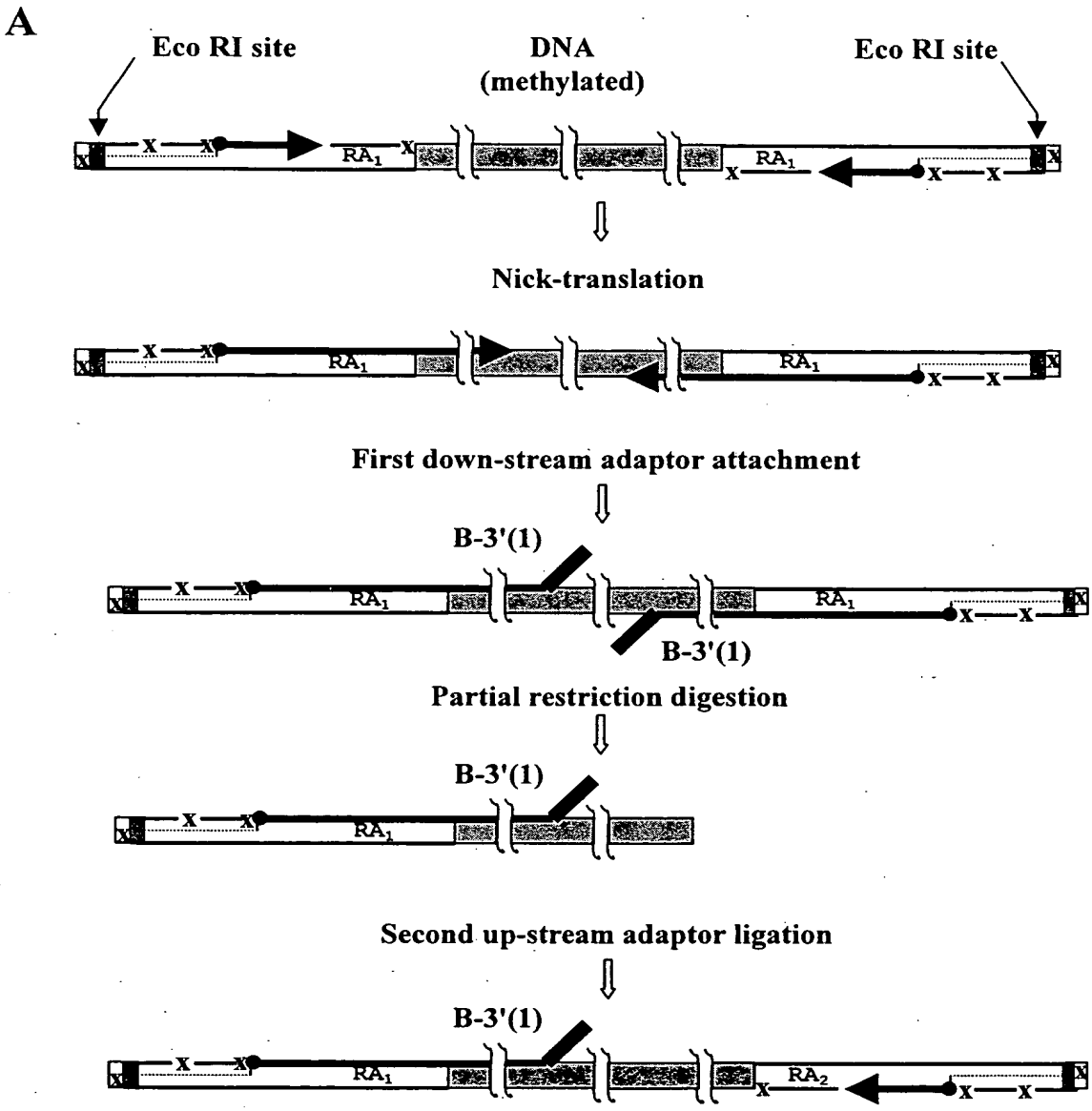

A
Eco RI site
DNA
(methylated)
Eco RI site
RA1
RA1
Nick-translation
RA1
RA1
First down-stream adaptor attachment
B-3'(1)
RA1
RA1
B-3'(1)
Partial restriction digestion
B-3'(1)
RA1
Second up-stream adaptor ligation
B-3'(1)
RA1
RA2

FIG. 31A

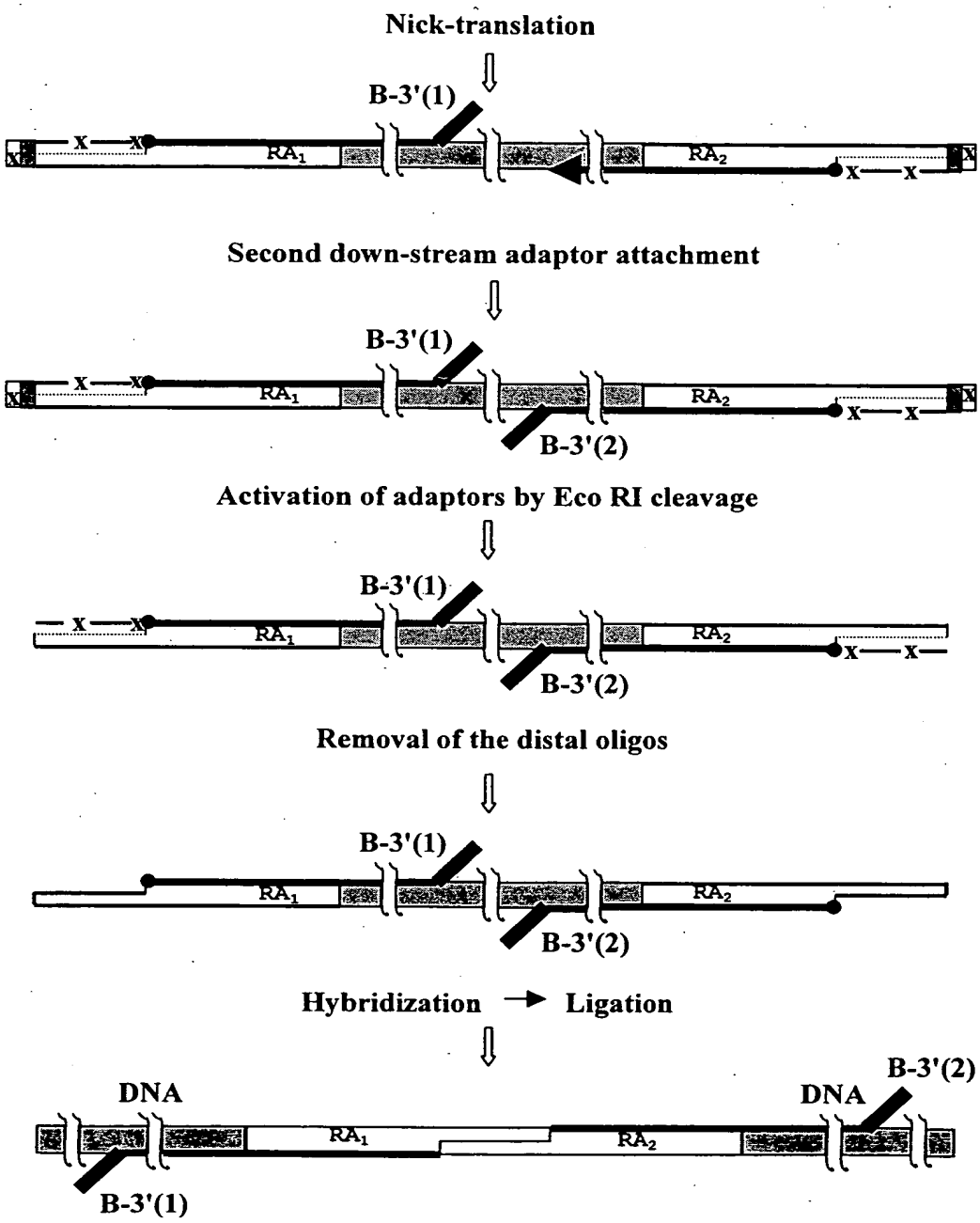

Nick-translation
B-3'(1)
RA1
RA2
Second down-stream adaptor attachment
B-3'(1)
B-3'(2)
Activation of adaptors by Eco RI cleavage
B-3'(1)
B-3'(2)
Removal of the distal oligos
B-3'(1)
B-3'(2)
Hybridization → Ligation
DNA
DNA
B-3'(2)
B-3'(1)

FIG. 31B

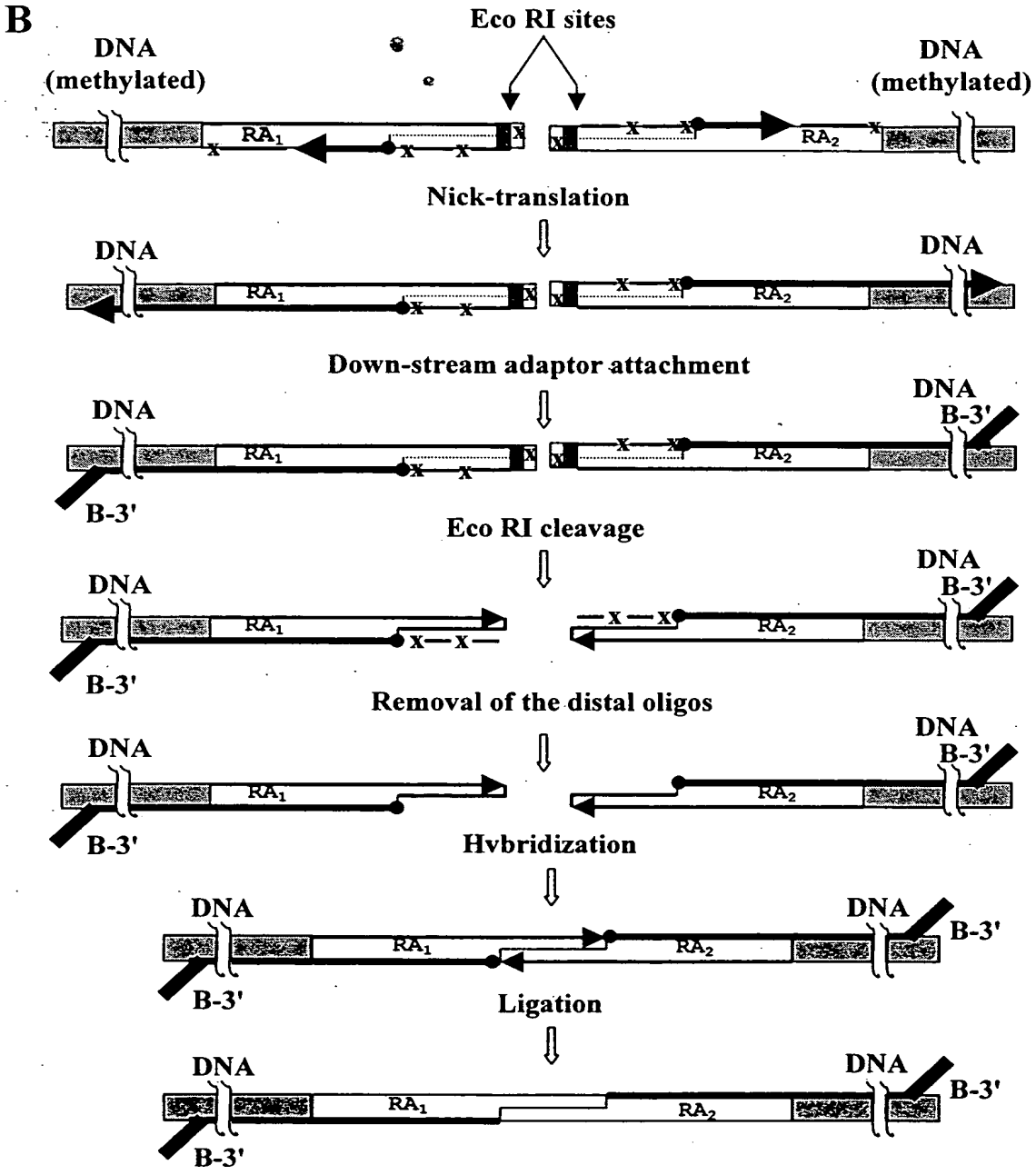

B
Eco RI sites
DNA
(methylated)
DNA
(methylated)
RA1
RA2
Nick-translation
DNA
DNA
Down-stream adaptor attachment
B-3'
Eco RI cleavage
Removal of the distal oligos
Hvbridization
Ligation

FIG. 31C

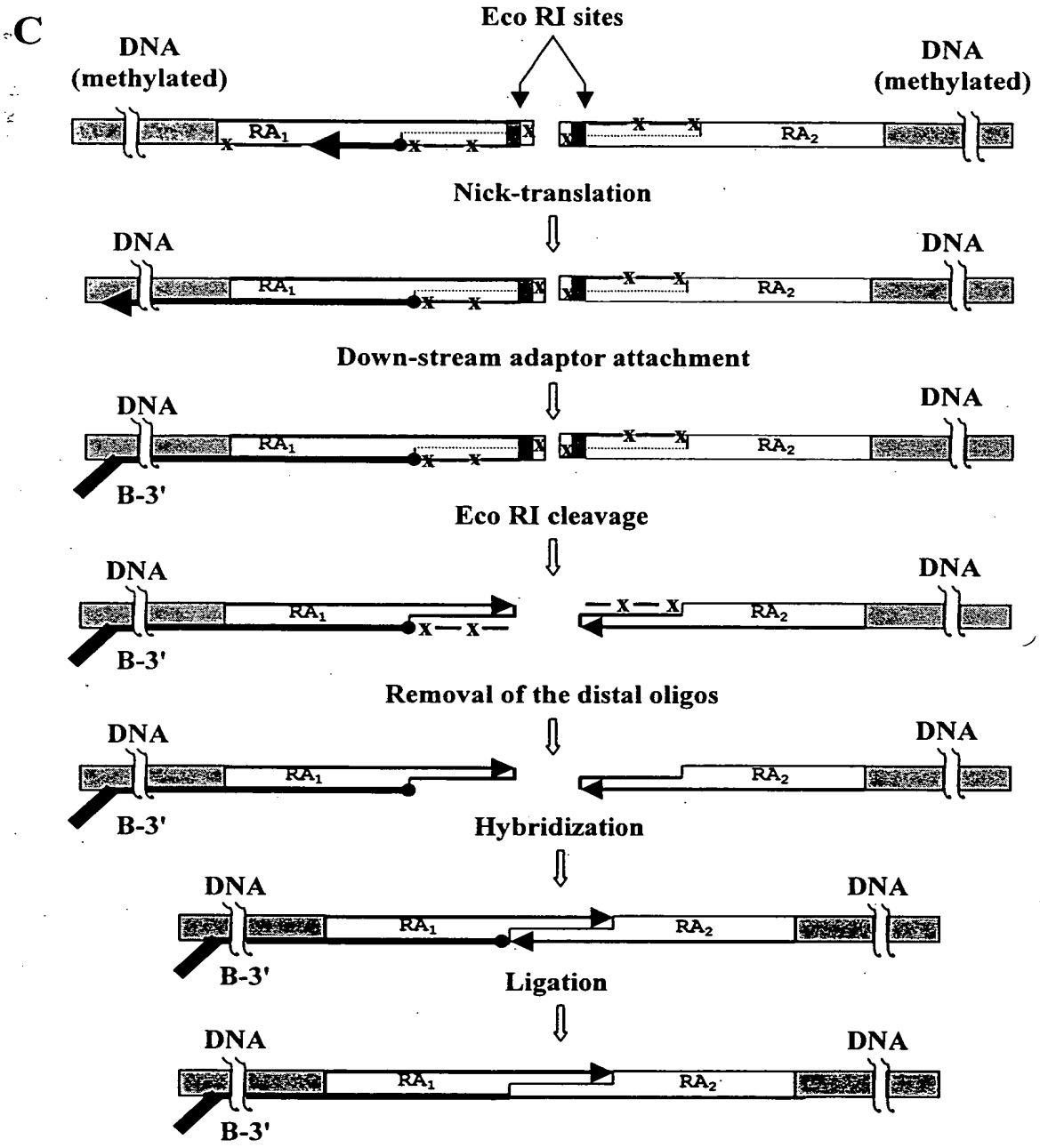

C
Eco RI sites
DNA
(methylated)
DNA
(methylated)
RA1
RA2
Nick-translation
DNA
DNA
RA1
RA2
Down-stream adaptor attachment
DNA
DNA
RA1
RA2
B-3'
Eco RI cleavage
DNA
DNA
RA1
RA2
B-3'
Removal of the distal oligos
DNA
DNA
RA1
RA2
B-3'
Hybridization
DNA
DNA
RA1
RA2
B-3'
Ligation
DNA
DNA
RA1
RA2
B-3'

FIG. 31D

D

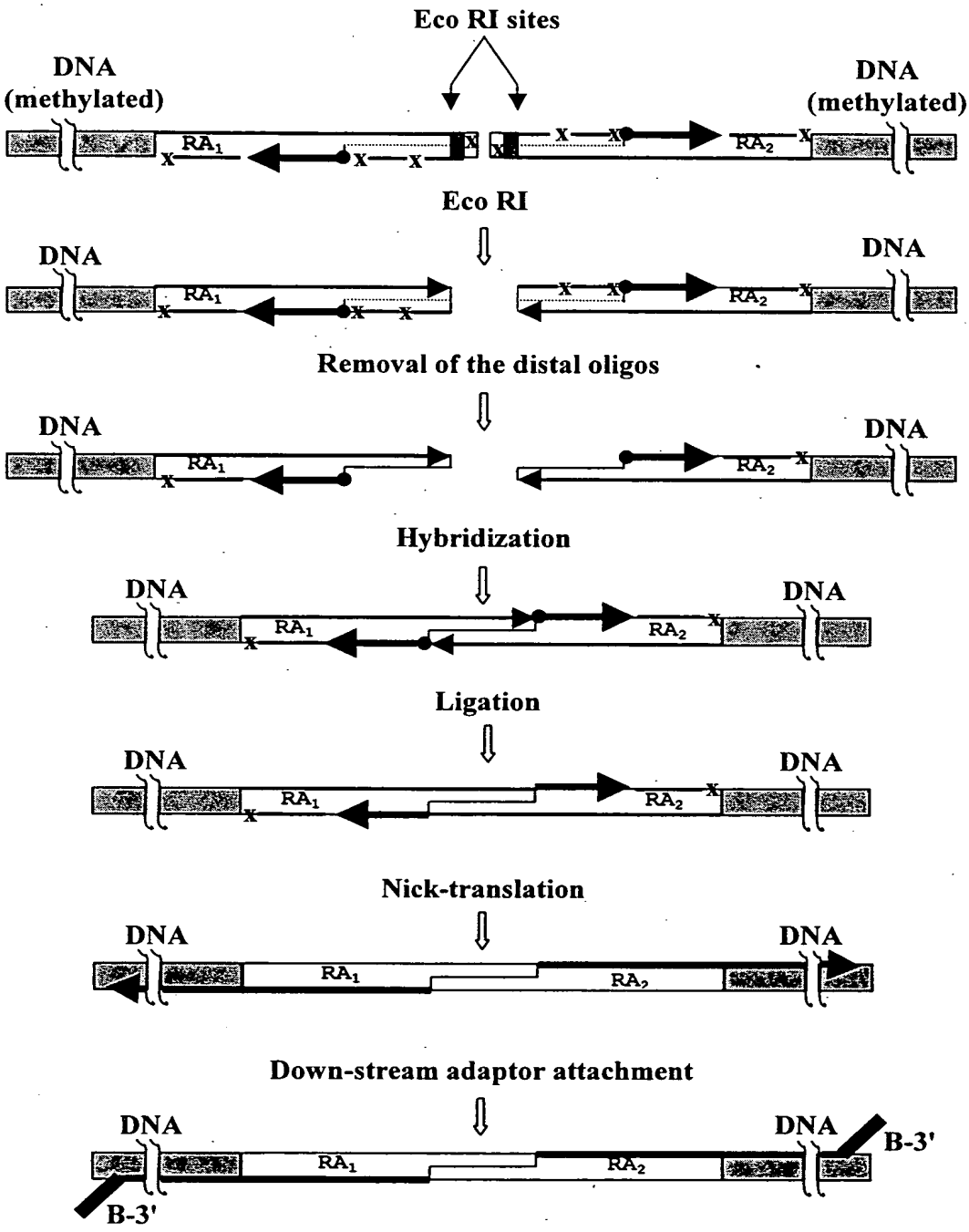

Eco RI sites
DNA
(methylated)
DNA
(methylated)
RA1
RA2
Eco RI
DNA
DNA
Removal of the distal oligos
Hybridization
Ligation
Nick-translation
Down-stream adaptor attachment
B-3'
B-3'

FIG. 31E

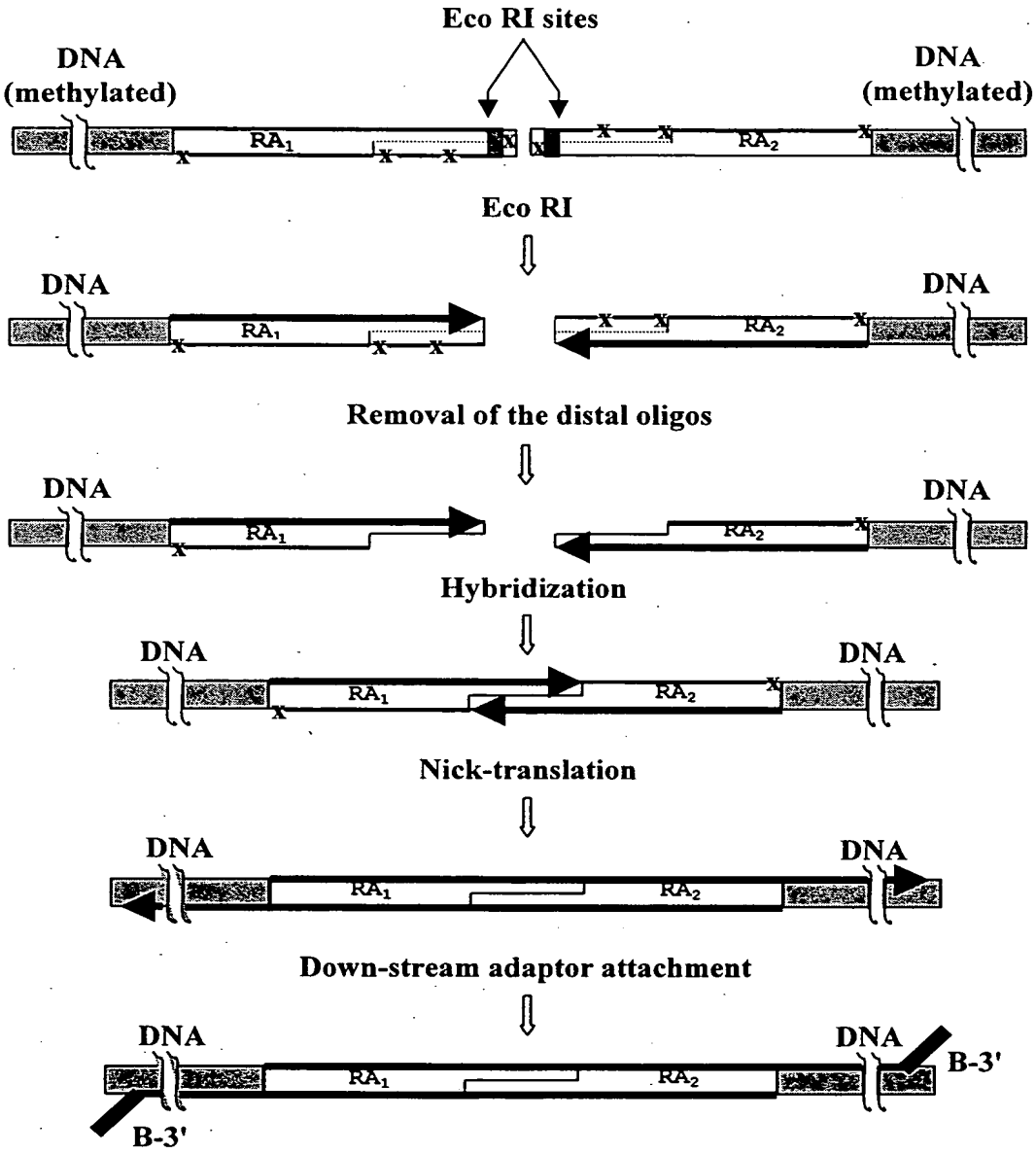

Eco RI sites
DNA
(methylated)
DNA
(methylated)
RA1
RA2
Eco RI
DNA
DNA
RA1
RA2
Removal of the distal oligos
DNA
DNA
RA1
RA2
Hybridization
DNA
DNA
RA1
RA2
Nick-translation
DNA
DNA
RA1
RA2
Down-stream adaptor attachment
DNA
DNA
B-3'
RA1
RA2
B-3'

FIG. 32

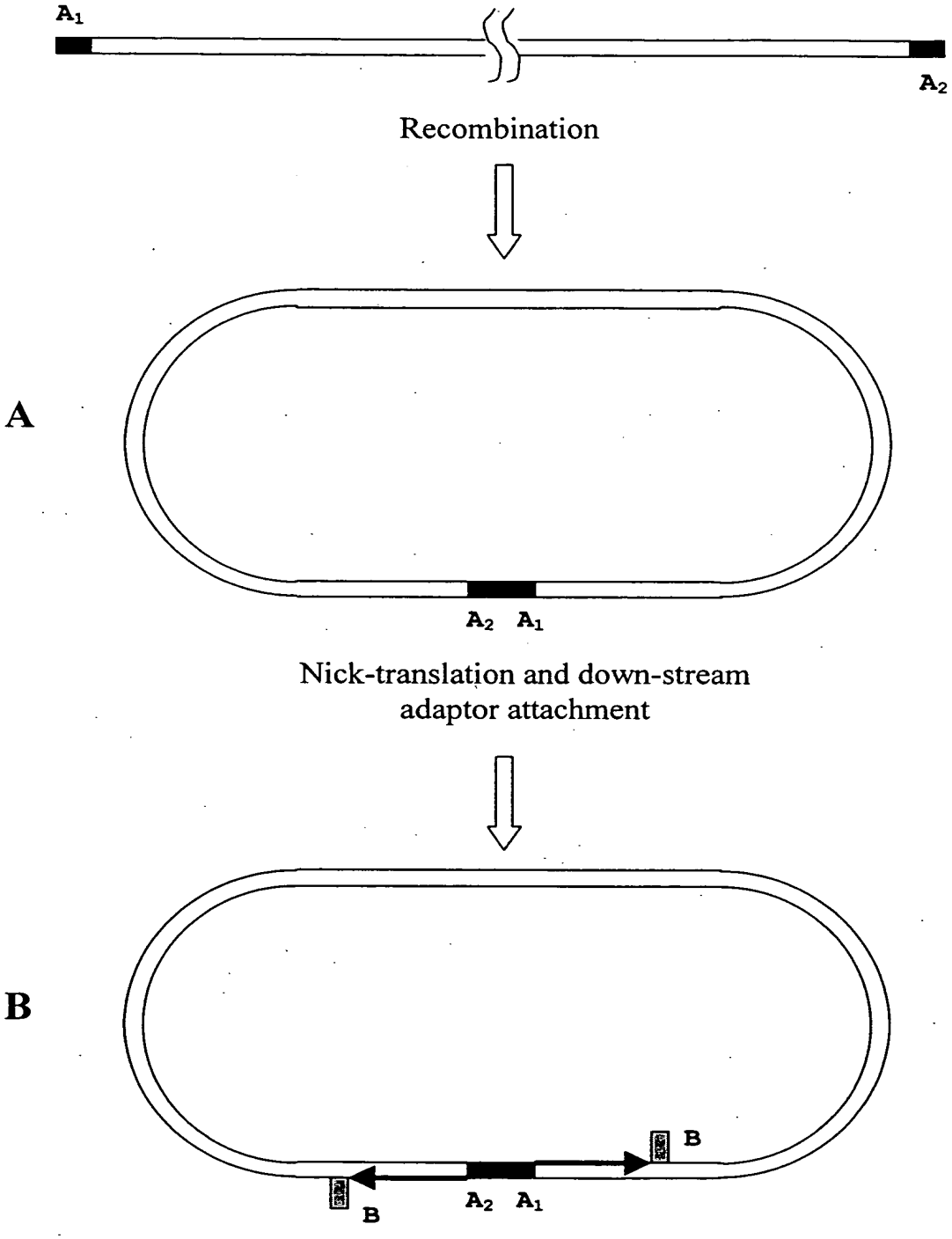

A1
A2
Recombination
A
A2 A1
Nick-translation and down-stream
adaptor attachment
B
B
B
A2 A1

FIG. 33

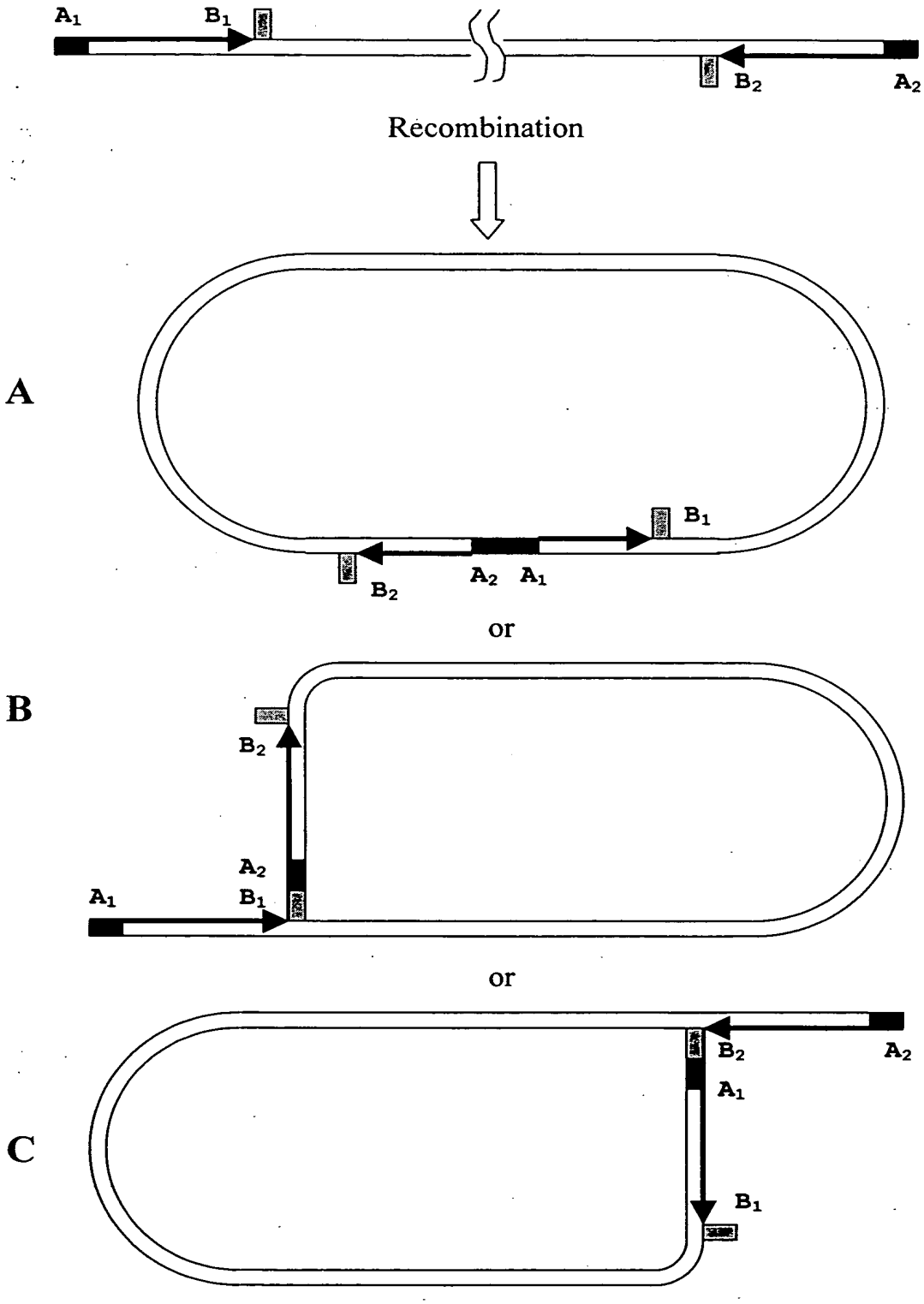
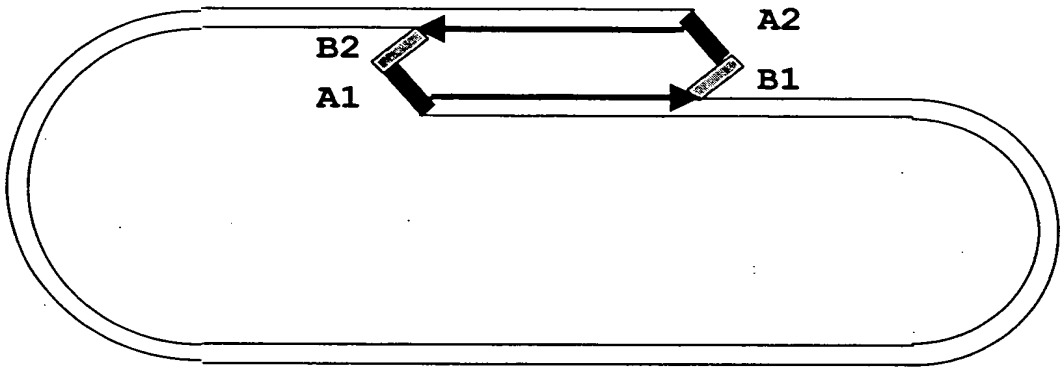

A1
B1
B2
A2
Recombination
A
B1
B2
A2
A1
or
B
B2
A2
A1
B1
or
C
B2
A2
A1
B1

FIG. 34A

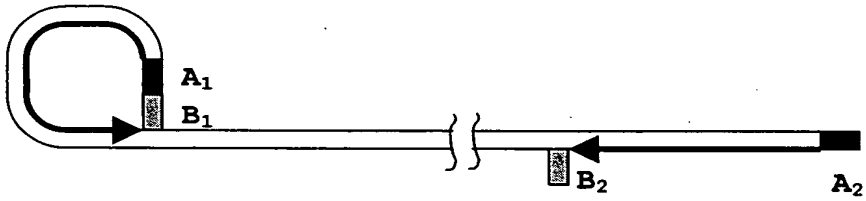
or

D



or

E



or

F

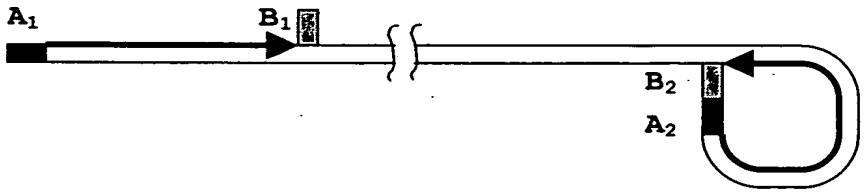


FIG. 34B

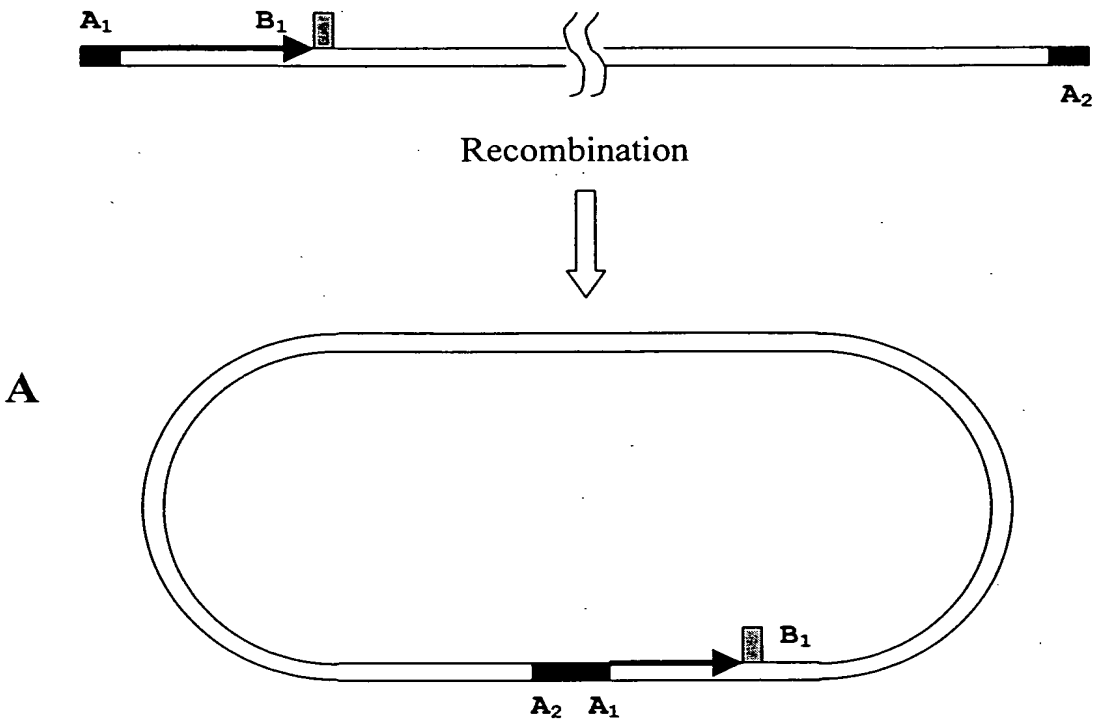


FIG. 35

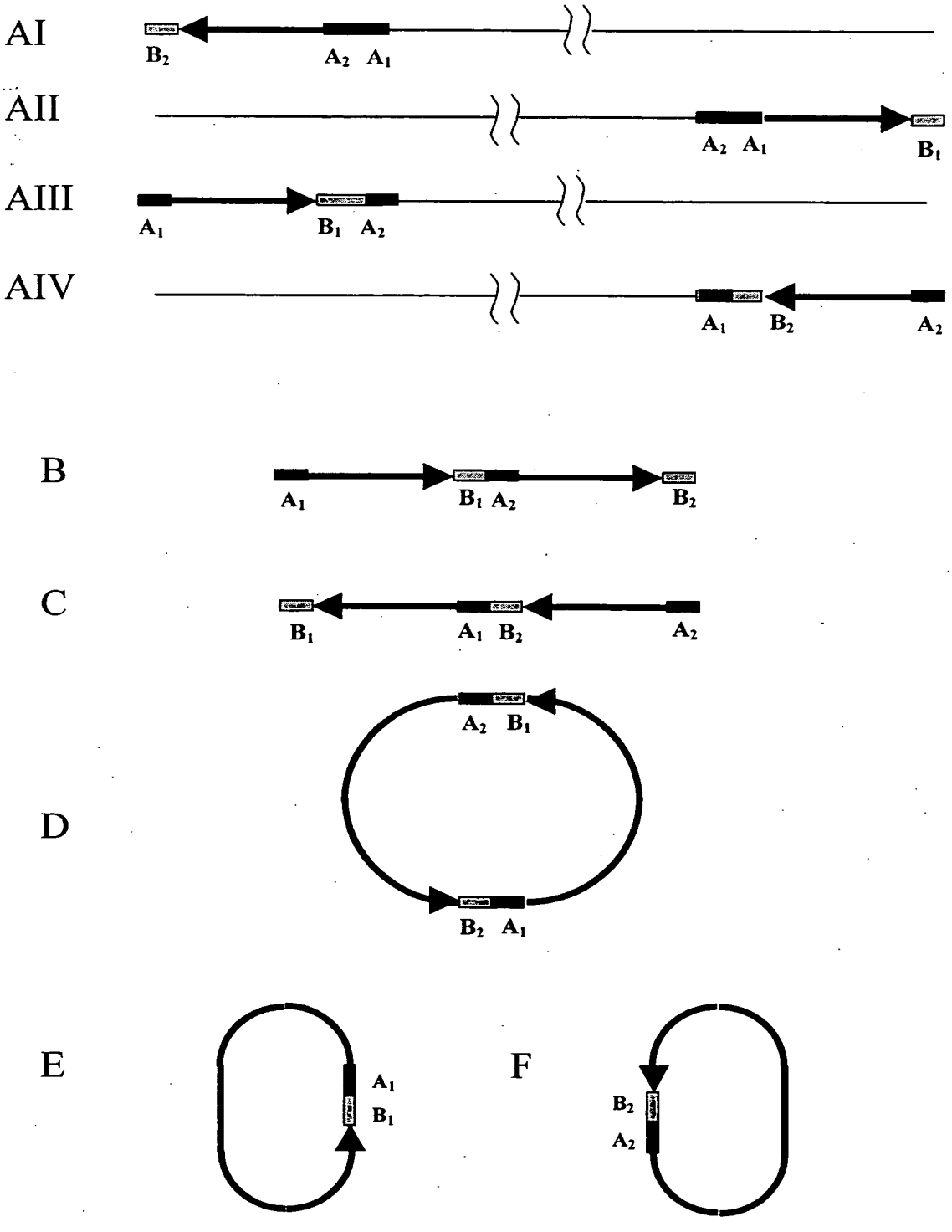
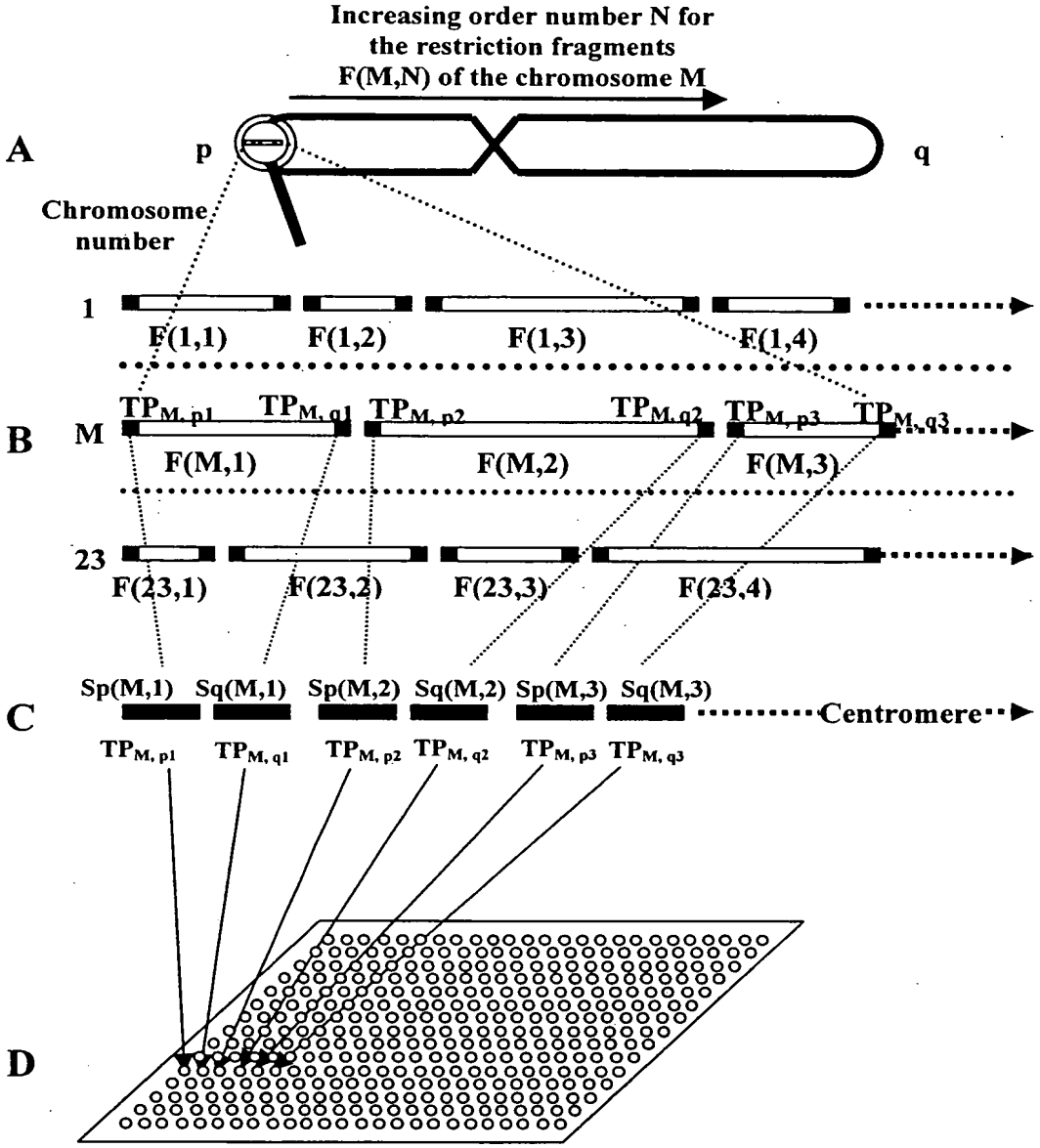

AI
B2
A2 A1
AII
A2 A1
B1
AIII
A1
B1 A2
AIV
A1 B2 A2
B
A1
B1 A2
B2
C
B1
A1 B2
A2
D
A2 B1
B2 A1
E
A1
B1
F
B2
A2

FIG. 36



Micro-array of ordered terminal PENTAmer (TP) DNA sequences

FIG. 37

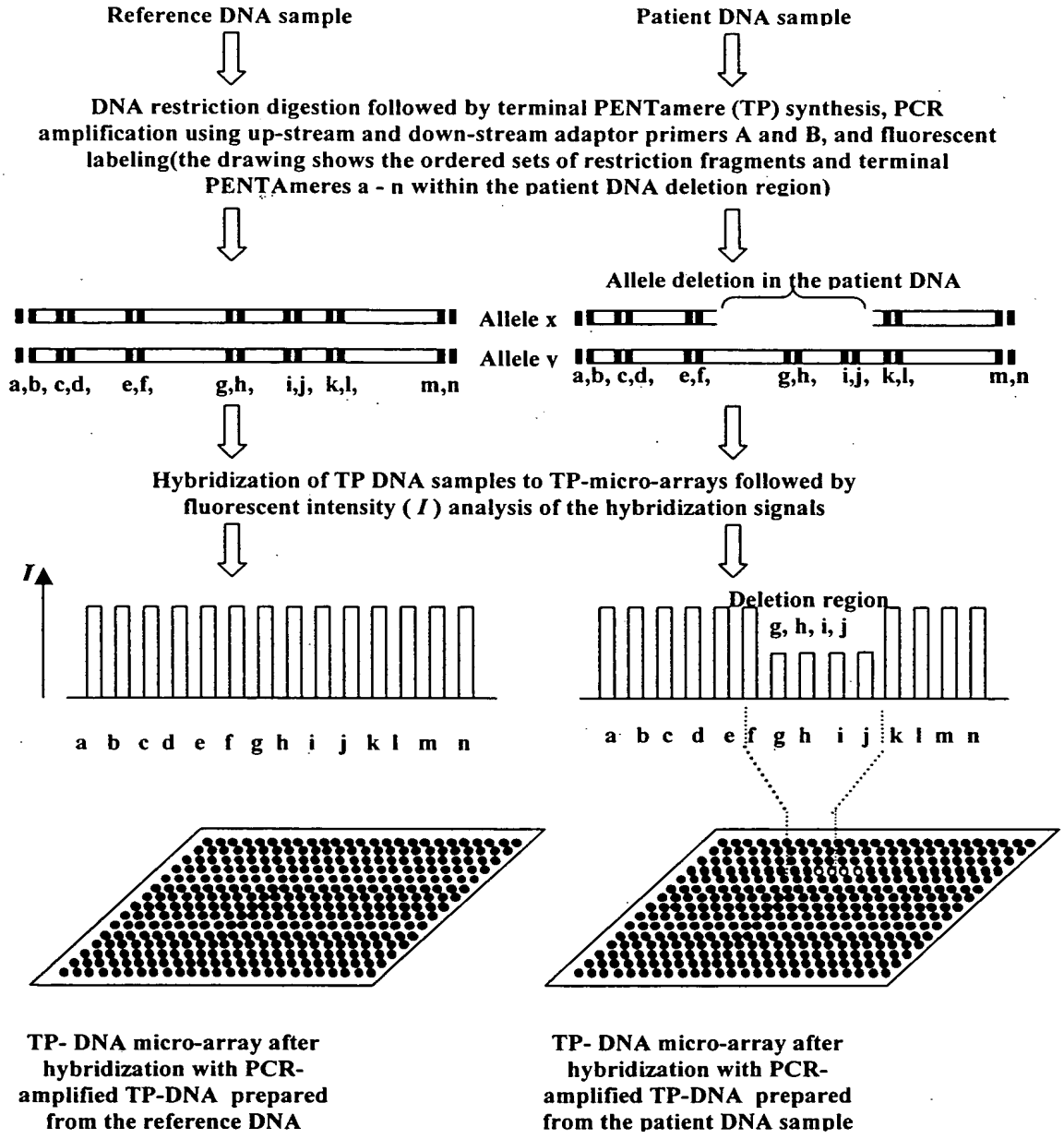

Reference DNA sample
Patient DNA sample
DNA restriction digestion followed by terminal PENTamere (TP) synthesis, PCR amplification using up-stream and down-stream adaptor primers A and B, and fluorescent labeling(the drawing shows the ordered sets of restriction fragments and terminal PENTAmeres a - n within the patient DNA deletion region)
Allele deletion in the patient DNA
Allele x
Allele y
a,b, c,d, e,f, g,h, i,j, k,l, m,n
a,b, c,d, e,f, g,h, i,j, k,l, m,n
Hybridization of TP DNA samples to TP-micro-arrays followed by fluorescent intensity (I) analysis of the hybridization signals
I
a b c d e f g h i j k l m n
Deletion region
g, h, i, j
a b c d e f g h i j k l m n
TP- DNA micro-array after hybridization with PCR-amplified TP-DNA prepared from the reference DNA
TP- DNA micro-array after hybridization with PCR-amplified TP-DNA prepared from the patient DNA sample

FIG. 38

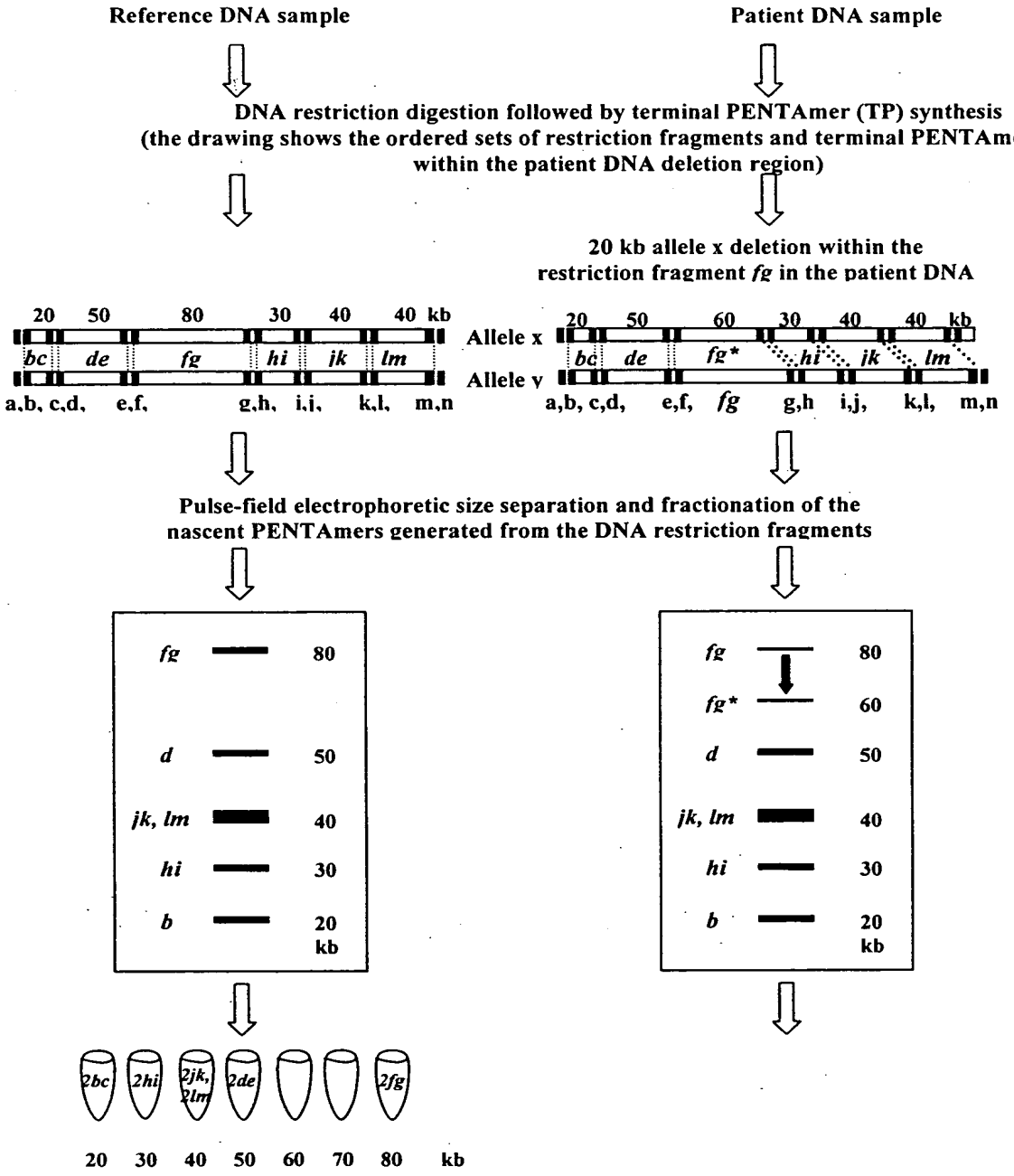
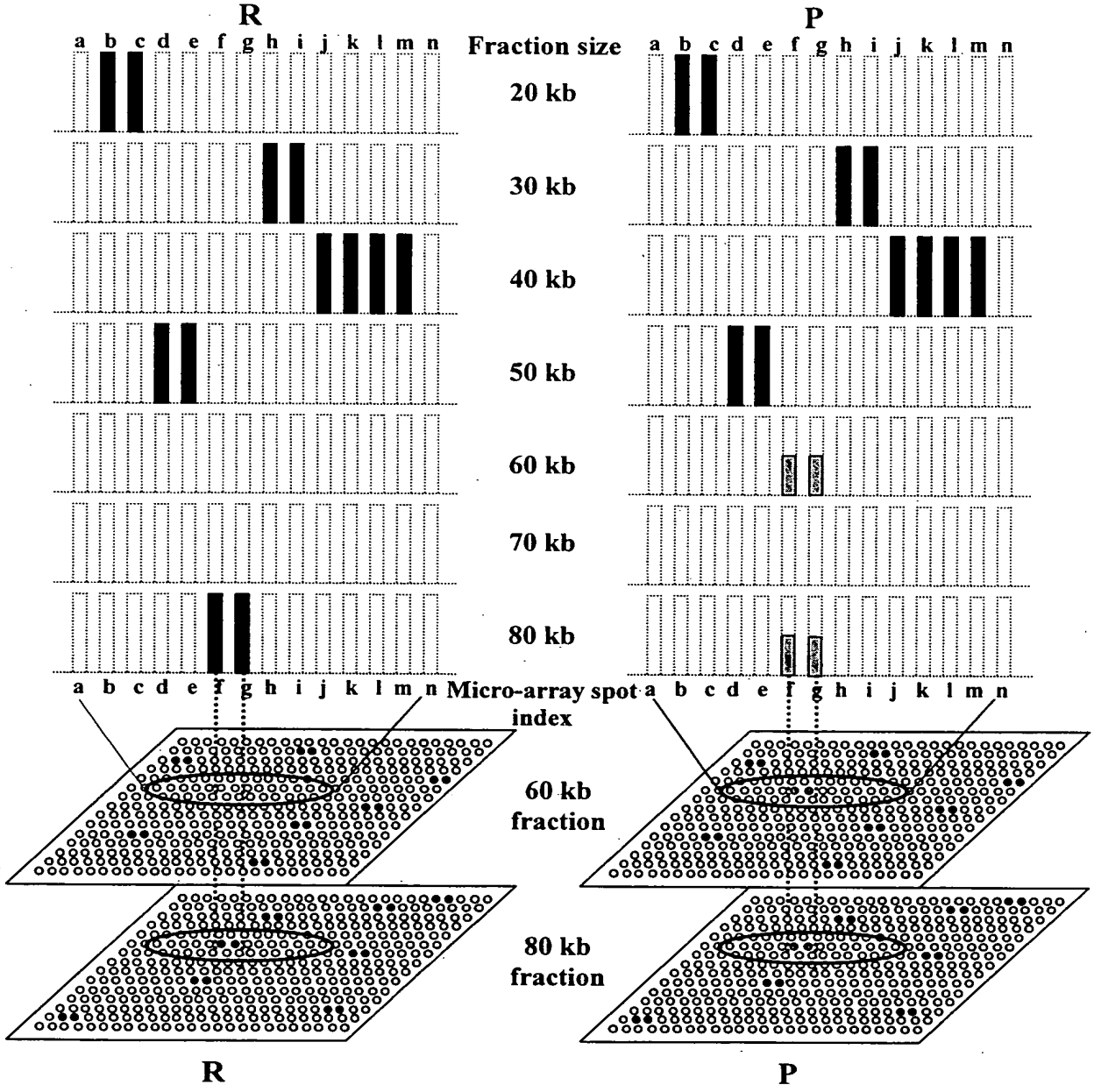

Reference DNA sample
Patient DNA sample
DNA restriction digestion followed by terminal PENTAmer (TP) synthesis
(the drawing shows the ordered sets of restriction fragments and terminal PENTAmers a - n within the patient DNA deletion region)
20 kb allele x deletion within the restriction fragment fg in the patient DNA
20 50 80 30 40 40 kb
bc de fg hi jk lm
a,b, c,d, e,f, g,h, i,j, k,l, m,n
Allele x
Allele y
20 50 60 30 40 40 kb
bc de fg* hi jk lm
a,b, c,d, e,f, fg g,h i,j, k,l, m,n
Pulse-field electrophoretic size separation and fractionation of the nascent PENTAmers generated from the DNA restriction fragments
fg 80
d 50
jk, lm 40
hi 30
b 20 kb
fg 80
fg* 60
d 50
jk, lm 40
hi 30
b 20 kb
2bc
2hi
2jk, 2lm
2de
2fg
20 30 40 50 60 70 80 kb

FIG. 39A

Hybridization of TP DNA size fractions to the TP-micro-array followed by fluorescent intensity analysis of the hybridization signals

Fluorescence intensity profiles of the TP- DNA micro-array a-n region after hybridization with PCR-amplified and labeled TP-DNA size fractions 20kb, 30kb, 40kb, 50kb, 60kb, 70kb and 80kb prepared from reference (R) and patient (P) DNA samples



TP- DNA micro-array a-n region after hybridization with PCR-amplified 60kb and 80 kb TP-DNA size fractions prepared from the reference (R) and patient (P) DNA samples

FIG. 39B

Up-stream, terminus-attaching, nick-translation adaptor A

5' **P** - GATCGCCTATACCTAGGACCATGTAA**ddC** 3' (SEQ ID NO. 16)
3' **dd**CGGAUATGGAUCCUGGUACATTG-OH 5' (SEQ ID NO. 17)

Acceptor-adaptor Ac

5' - GATCGCCTATACCTAGGACCATGTAA 3' (SEQ ID NO. 18)
3' CGGAUATGGAUCCUGGUACATTG-OH 5' (SEQ ID NO. 19)

Recombination, nick-translation adaptor RA-(*L-cos*)

5' **P** - GATCGCCTATACCTAGGACCATGTAAC**GAATTC**ATCA 3' (SEQ ID NO. 20)
NH_2CGGAUATGGAUCCTGGUACATUG**CTTAAG**TAGTCCCGCCGCTGGA-OH 5' (SEQ ID NO. 21)

Down-stream, nick-attaching adaptors B-3' (a), B-3' (b), B-3' (c) and B-3' (d)

5'-GGGAGATCTGAATTCCCCCCCCCCC**ddC**-3' (SEQ ID NO. 22)
3' -**dd**CGCCACTGGGCCCTCTAGACTTAAG - **P** 5'(SEQ ID NO. 23) (a)

5'-GTTACATGGTCCTAGGTATAGGC GCGGTGACCCGGGAGATCTGCCCCCCCCCC-3' (SEQ ID NO. 24)
3'- AATGTACCAGGATCCATATCCGCGCCACTGGGCCCTCTAGAC - **P** 5' (SEQ ID NO. 25) (b)

5' -GGGAGATTCTGAATTCAAAAAAAA**ddA**-3' (SEQ ID NO. 26)
3' -**dd**AGCCACTGGGCCCTCTAGACTTAAG - **P** 5' (SEQ ID NO. 27) (c)

5-GTTACATGGTCCTAGGTATAGGC GCGGTGACCCGGGAGATCTGAAAAAAAAAA-3' (SEQ ID NO. 28)
3- AATGTACCAGGATCCATATCCGCGCCACTGGGCCCTCTAGAC - **P** 5' (SEQ ID NO. 29) (d)

Oligo-construct with nick ^{32}**P-A** nick

5'-Biotin-GCGGTGACCCGGGAGATCTGAATTCA GGGCGGCGACCT-3' (SEQ ID NO. 30 & 31)
3'- CGCCACTGGGCCCTCTAGACTTAAGTCCCGCCGCTGGA - **P**-5' (SEQ ID NO. 32)

a) for a nomenclature of the adaptors A and B-3' see section 6

Fig. 40

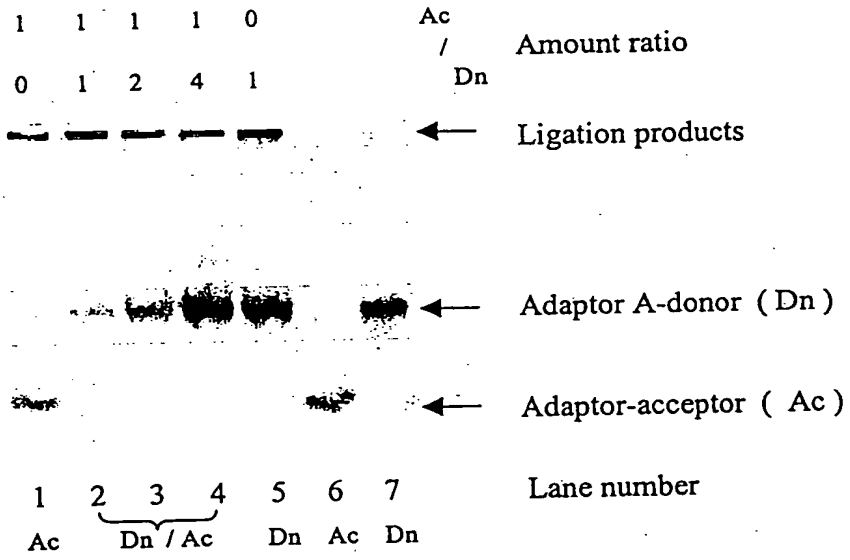

1 1 1 1 0
0 1 2 4 1
Ac / Dn
Amount ratio
Ligation products
Adaptor A-donor (Dn)
Adaptor-acceptor (Ac)
1 2 3 4 5 6 7
Ac Dn / Ac Dn Ac Dn
Lane number

Fig. 41

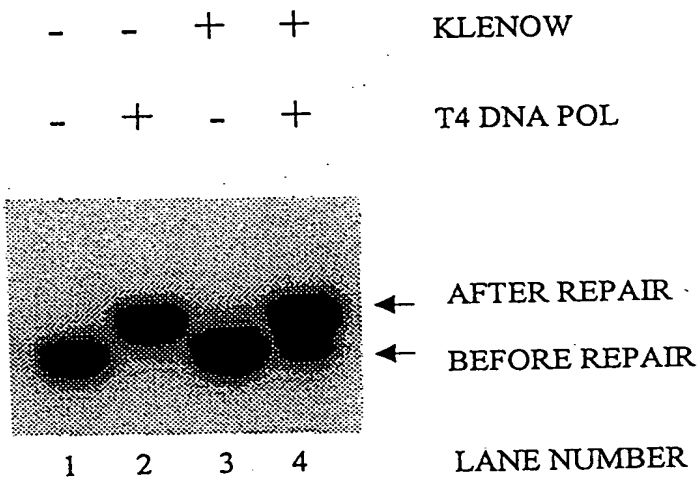

- - + + KLENOW
- + - + T4 DNA POL
AFTER REPAIR
BEFORE REPAIR
1 2 3 4 LANE NUMBER

Fig. 42

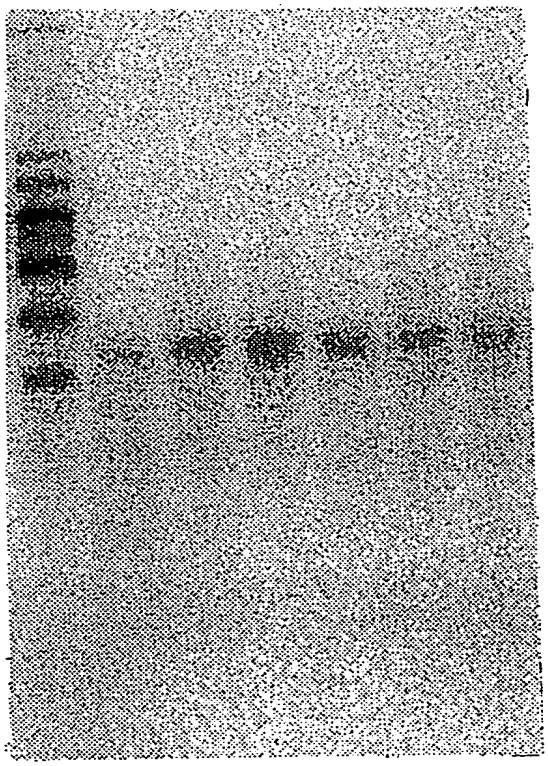


Fig. 43

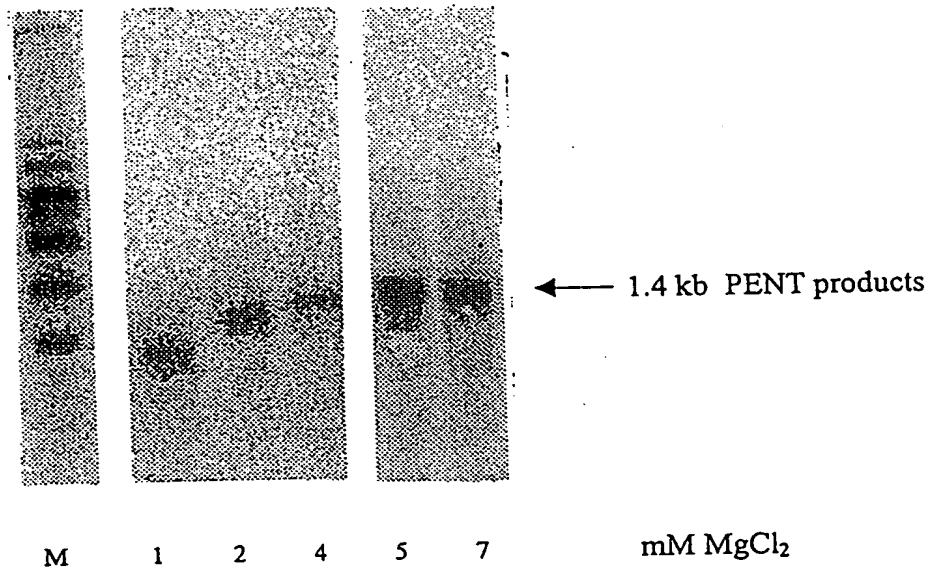


Fig. 44

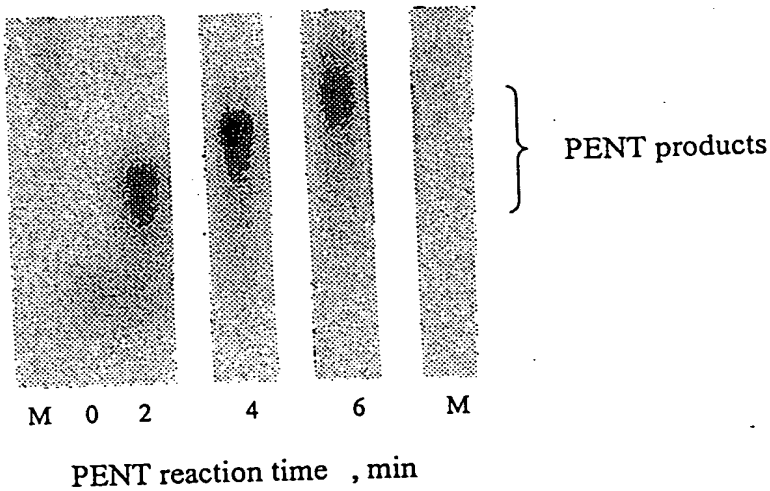


Fig. 45

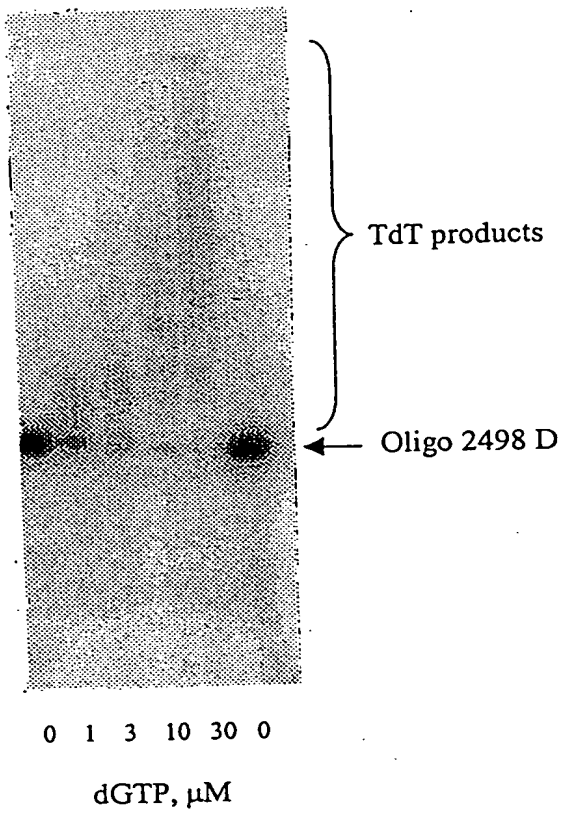


Fig. 46

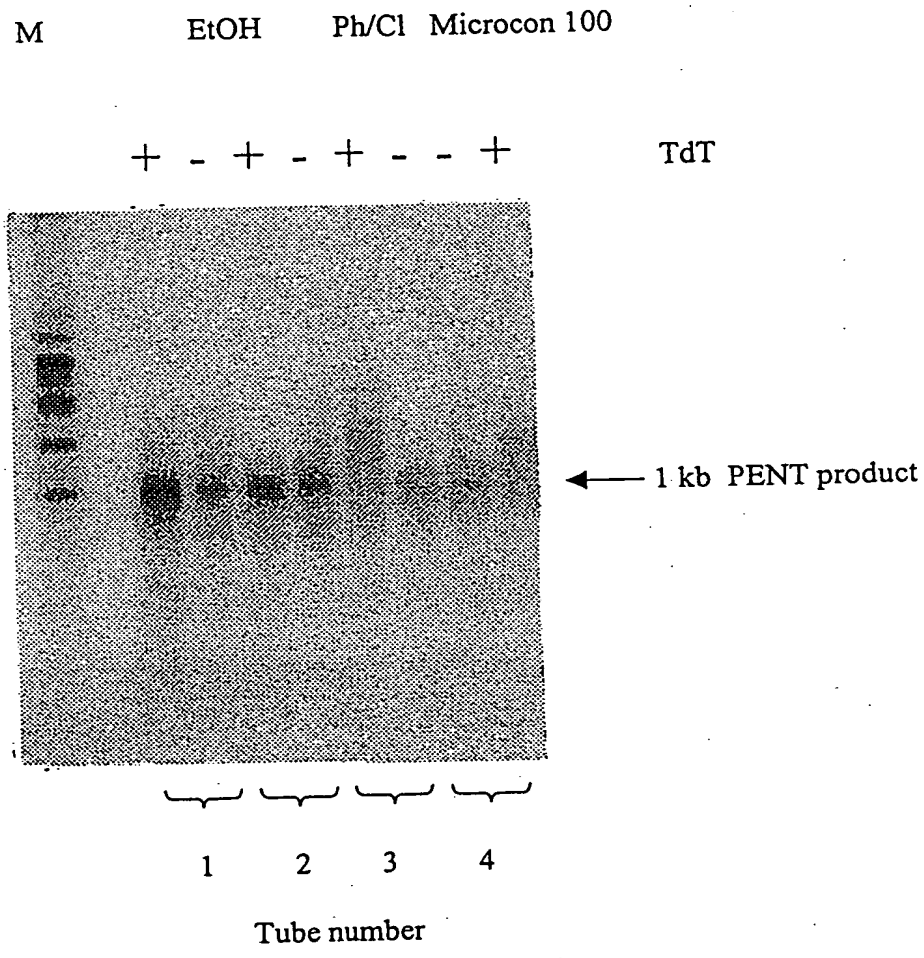

M
EtOH
Ph/Cl
Microcon 100
+ - + - + - - +
TdT
1 kb PENT product
1
2
3
4
Tube number

Fig. 47

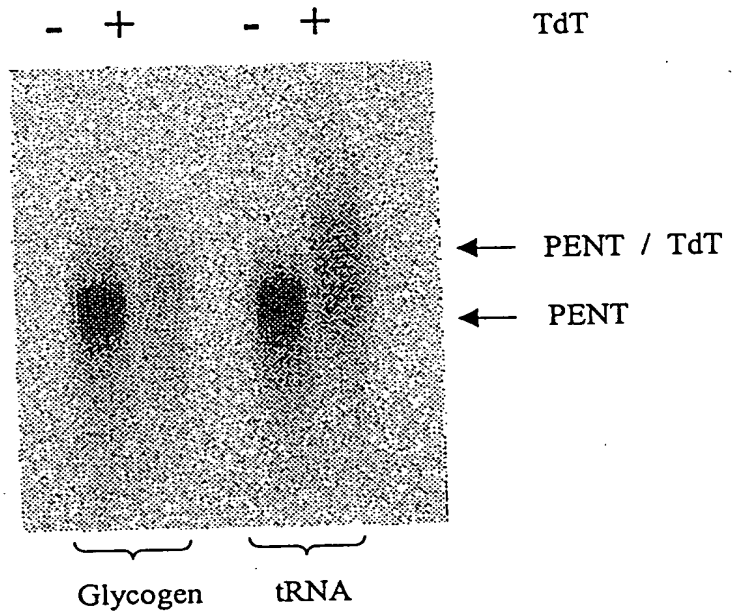


Fig. 48

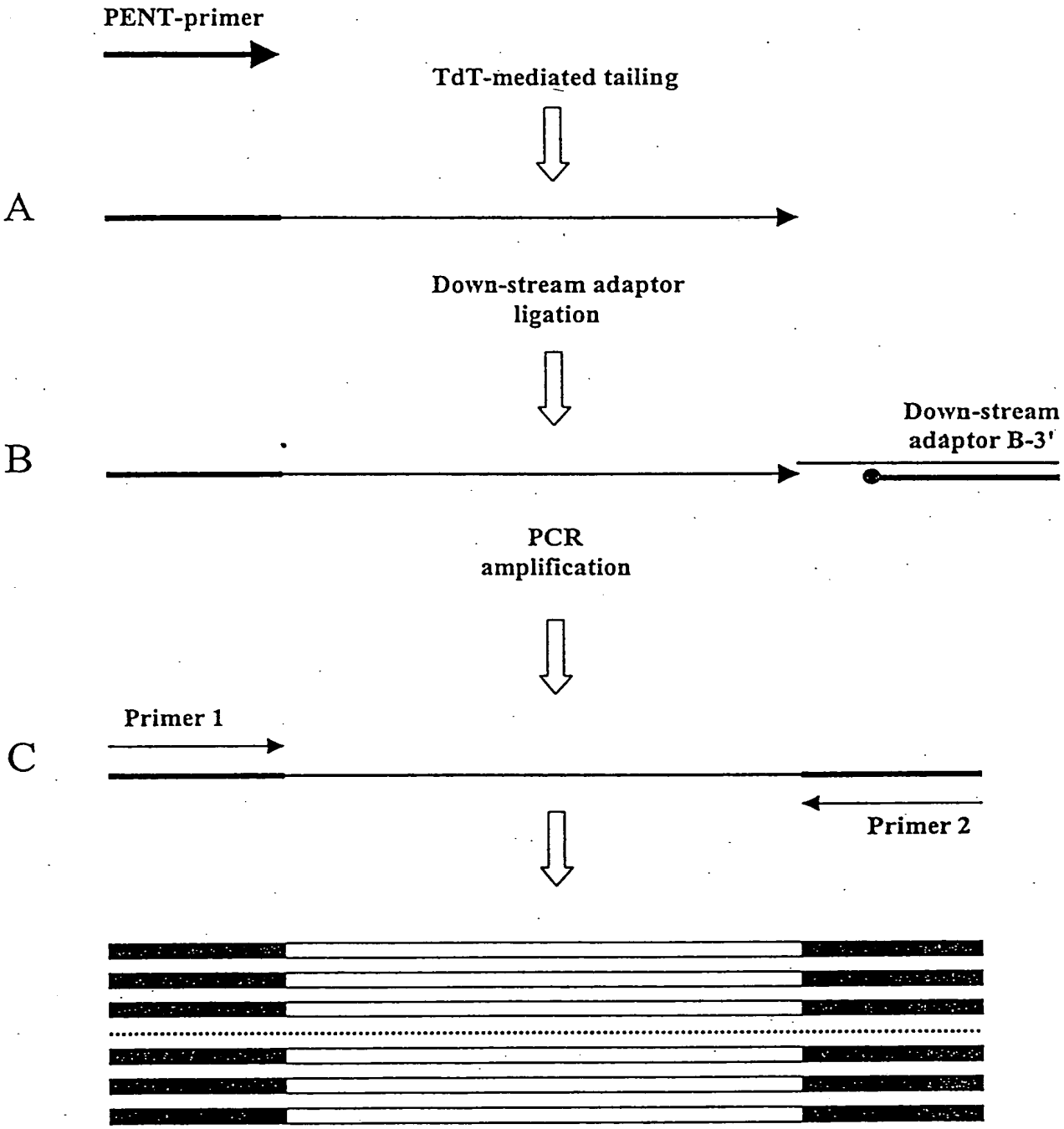

PENT-primer
TdT-mediated tailing
A
Down-stream adaptor ligation
Down-stream adaptor B-3'
B
PCR amplification
Primer 1
C
Primer 2

Fig. 49

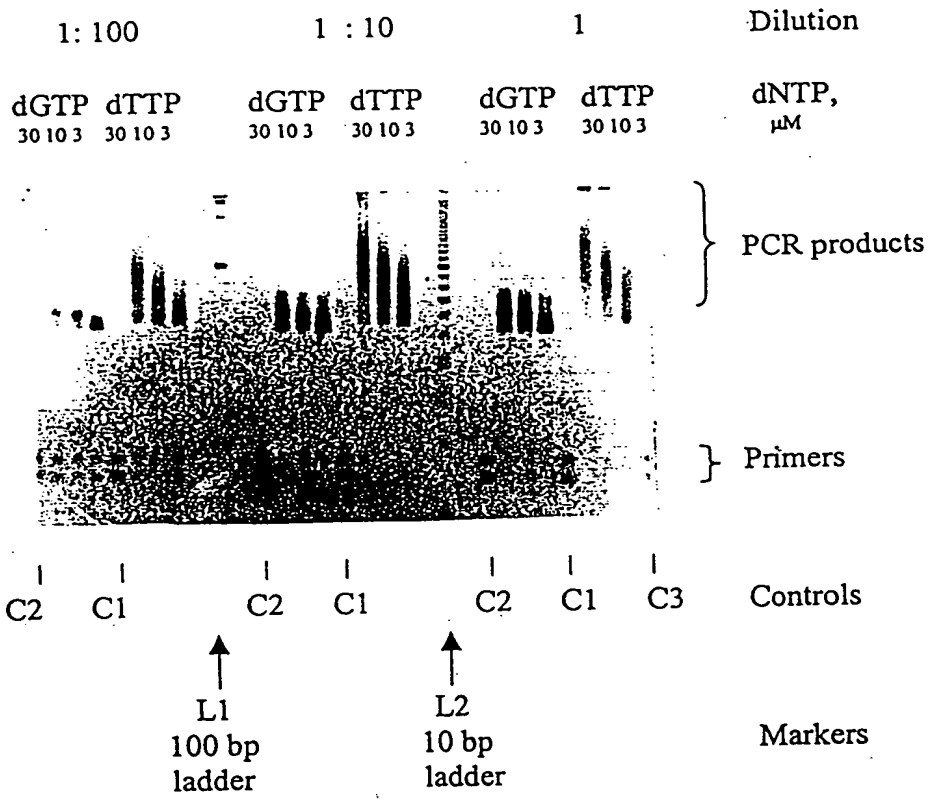

1: 100
1 : 10
1
Dilution
dGTP dTTP
30 10 3 30 10 3
dGTP dTTP
30 10 3 30 10 3
dGTP dTTP
30 10 3 30 10 3
dNTP, μM
PCR products
Primers
C2 C1 C2 C1 C2 C1 C3
Controls
L1
100 bp
ladder
L2
10 bp
ladder
Markers

Fig. 50

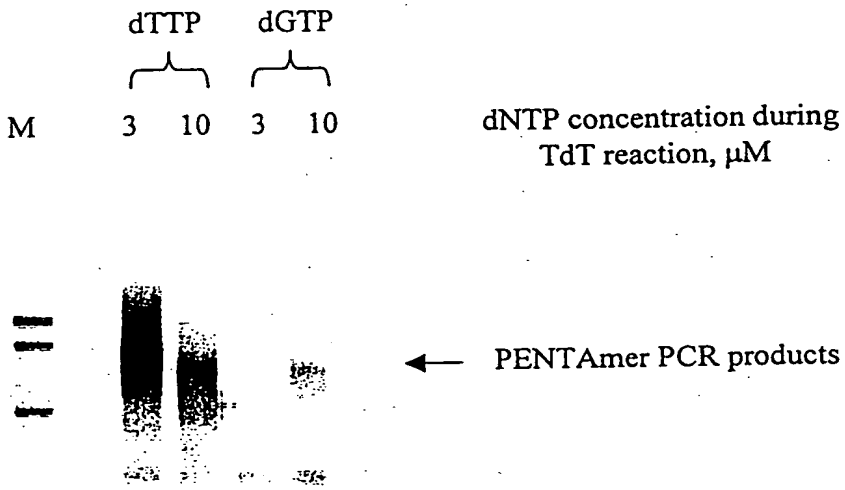

dTTP
dGTP
M
3
10
3
10
dNTP concentration during TdT reaction, μM
PENTAmer PCR products

Fig. 51

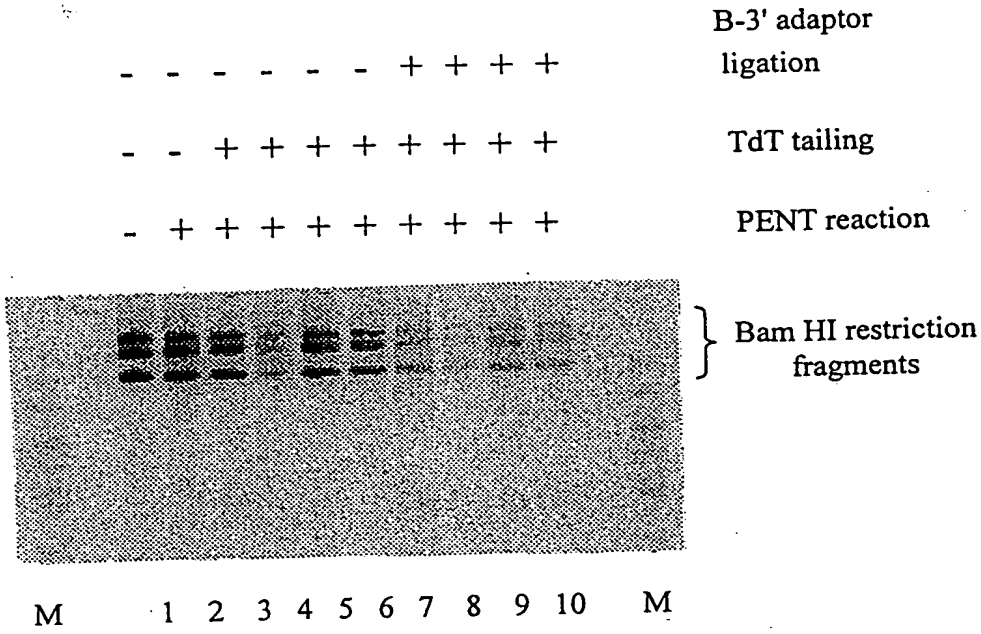

B-3' adaptor ligation
- - - - - - + + + +
TdT tailing
- - + + + + + + + +
PENT reaction
- + + + + + + + + +
Bam HI restriction fragments
M 1 2 3 4 5 6 7 8 9 10 M

Fig. 52

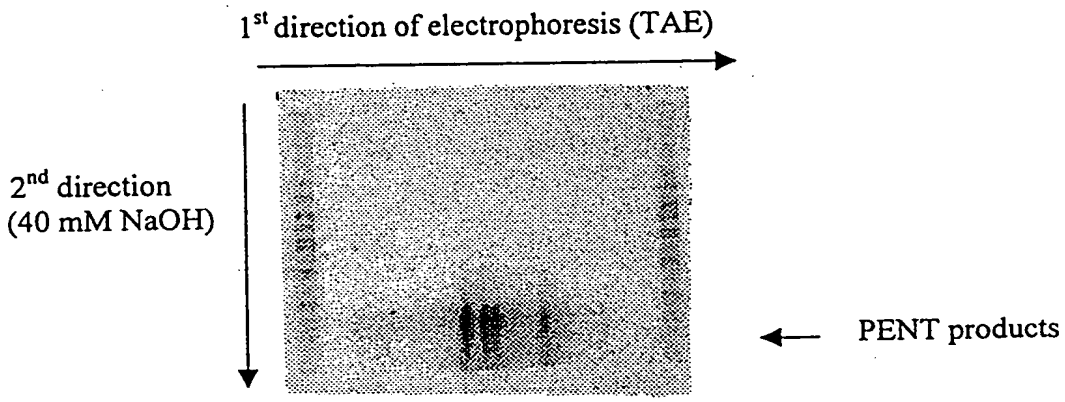

1st direction of electrophoresis (TAE)
2nd direction
(40 mM NaOH)
PENT products

Fig. 53

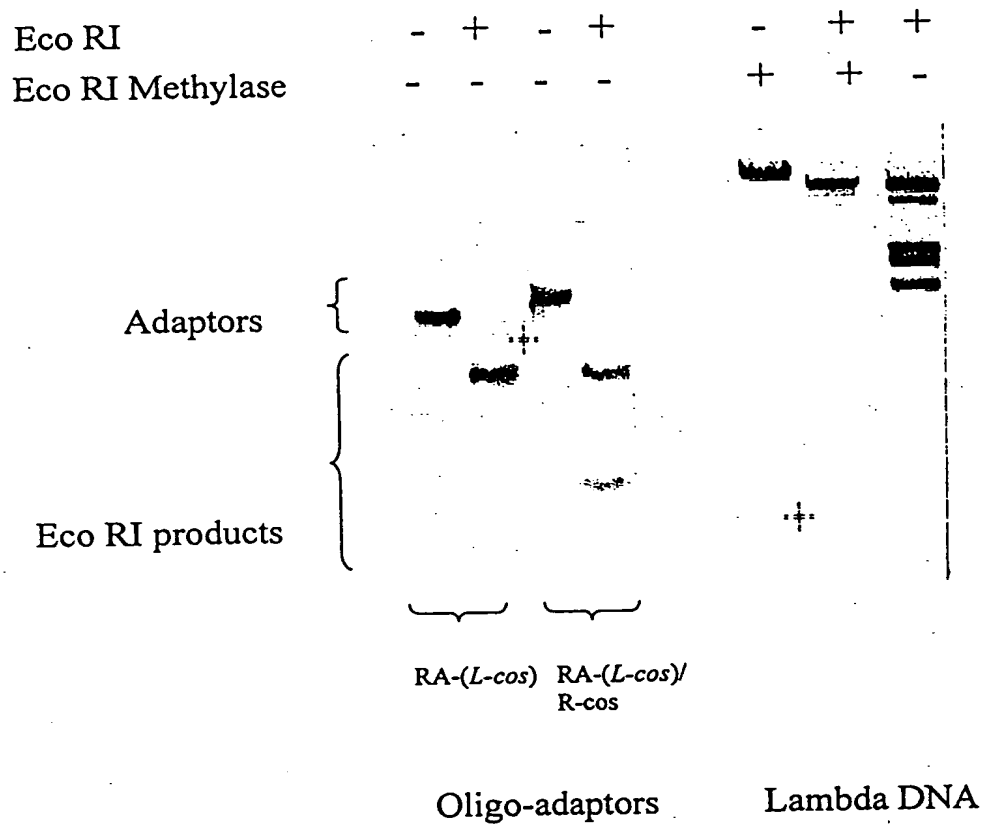

Eco RI
Eco RI Methylase
- + - +
- - - -
- + +
+ + -
Adaptors
Eco RI products
RA-(L-cos)
RA-(L-cos)/
R-cos
Oligo-adaptors
Lambda DNA

Fig. 54

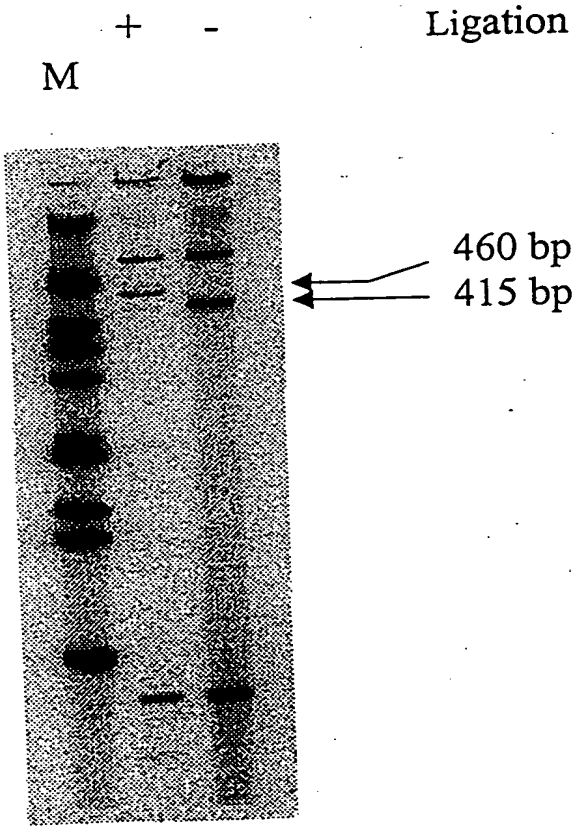


Fig. 55

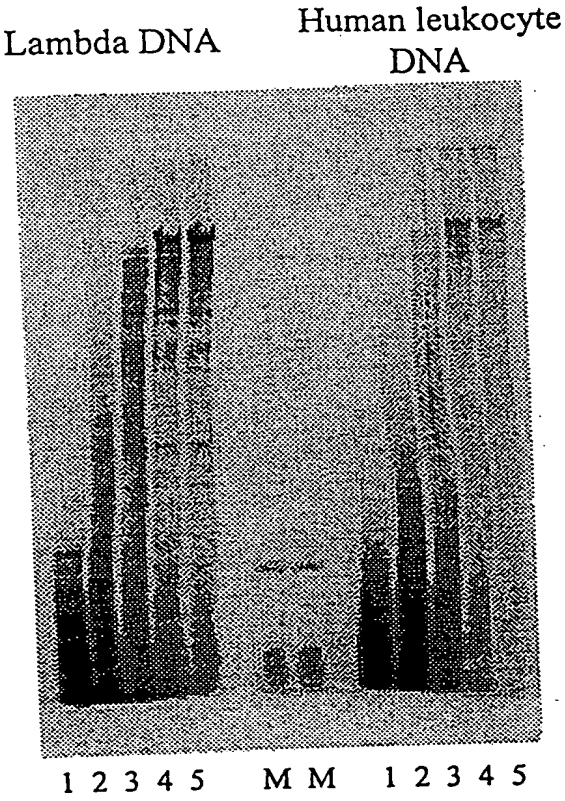


Fig. 56

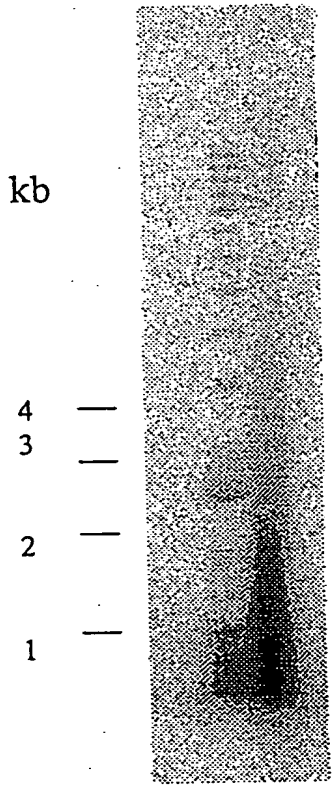


Fig. 57

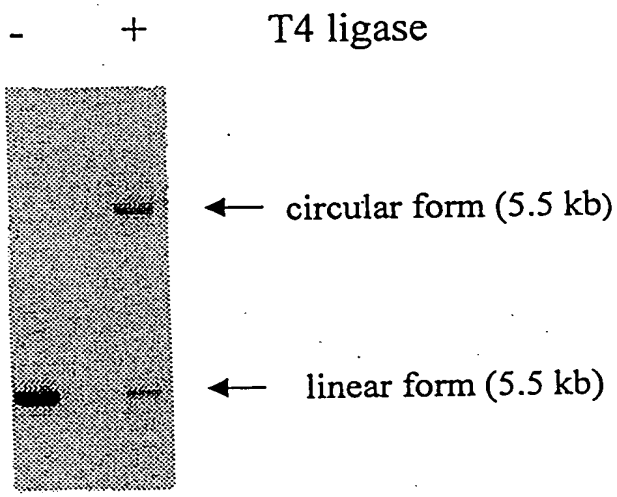


Fig. 58

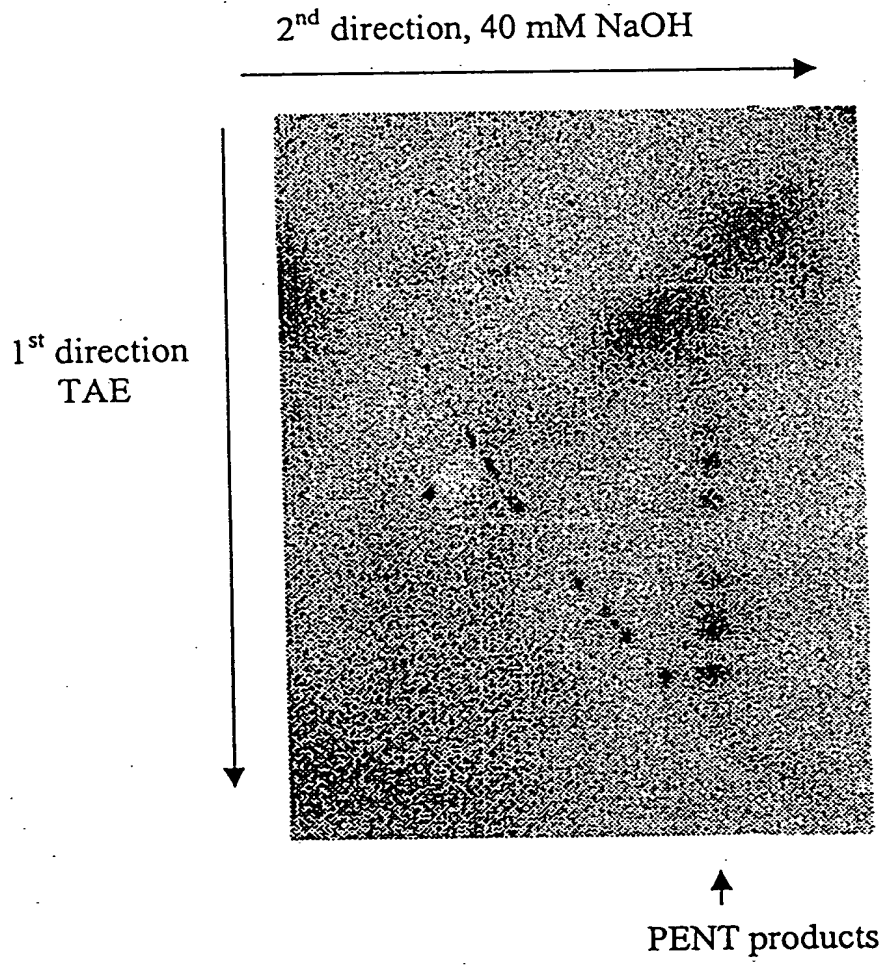


Fig. 59

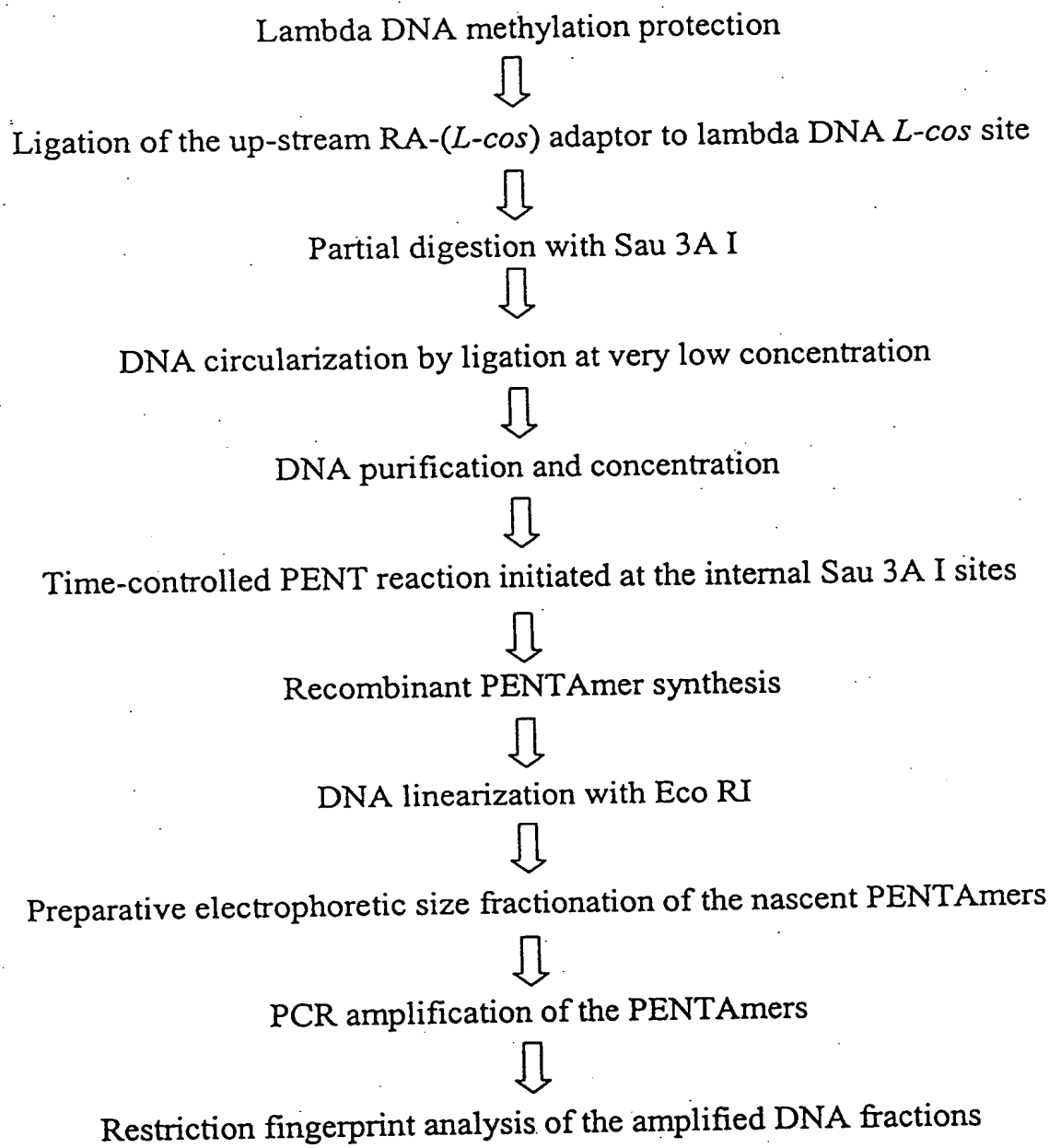
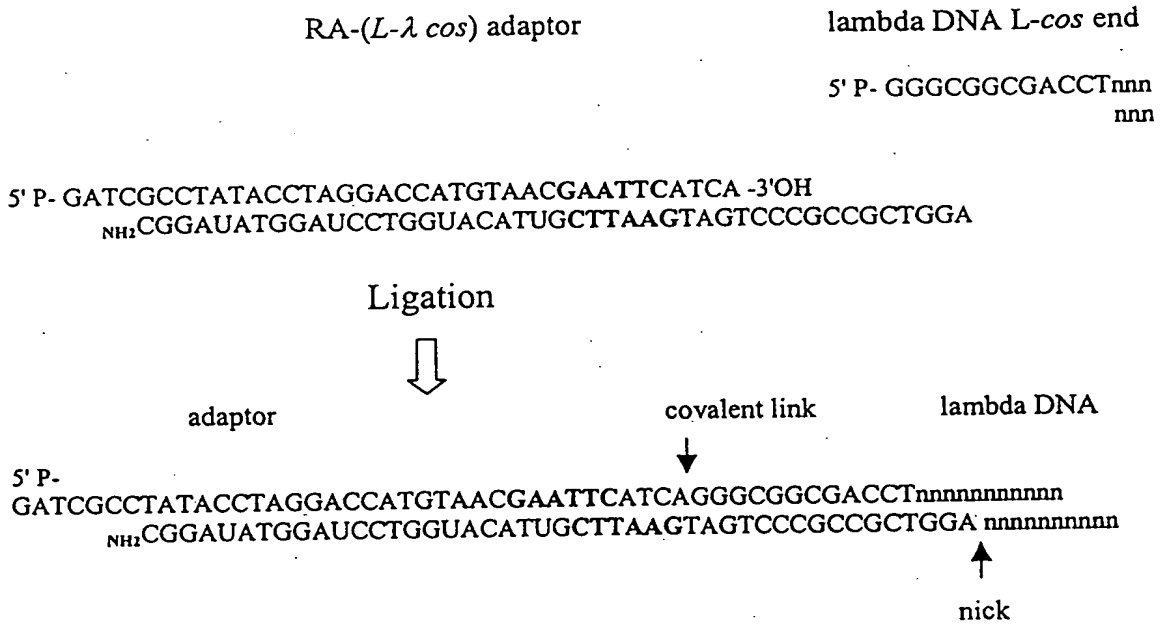
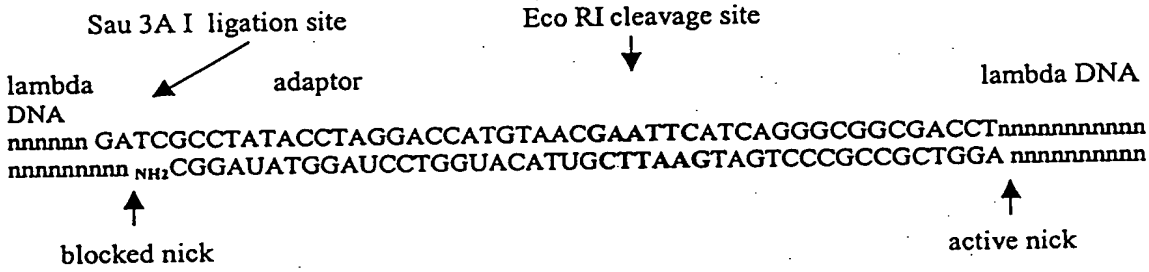

Lambda DNA methylation protection
Ligation of the up-stream RA-(*L-cos*) adaptor to lambda DNA *L-cos* site
Partial digestion with Sau 3A I
DNA circularization by ligation at very low concentration
DNA purification and concentration
Time-controlled PENT reaction initiated at the internal Sau 3A I sites
Recombinant PENTAmer synthesis
DNA linearization with Eco RI
Preparative electrophoretic size fractionation of the nascent PENTAmers
PCR amplification of the PENTAmers
Restriction fingerprint analysis of the amplified DNA fractions

Fig. 60

A The RA-(*L-λ cos*) adaptor - Lambda DNA junction structure



B The recombinant junction formed by a circularization reaction .



C The 5' -end of the PENTAmer

nnnnnnnnnctagCGGAUATGGAUCCTGGUACATUGCTTAA - 5'

Fig. 61

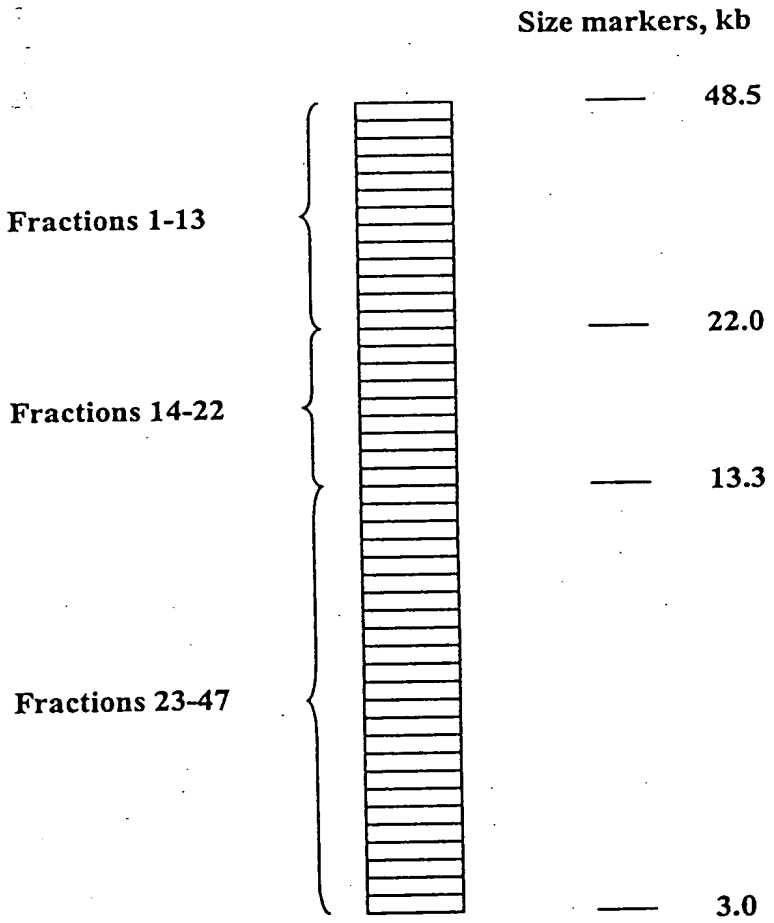
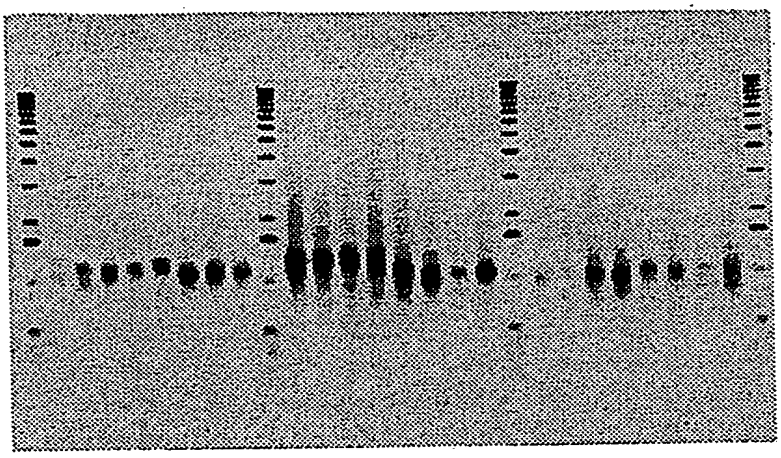
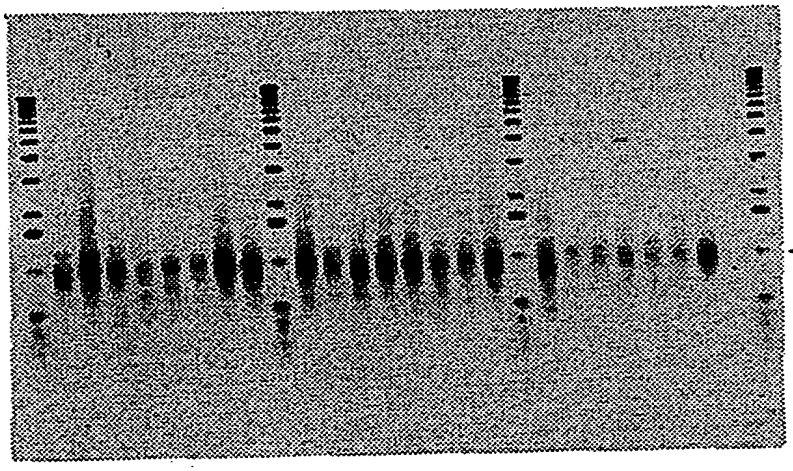

Size markers, kb
48.5
22.0
13.3
3.0
Fractions 1-13
Fractions 14-22
Fractions 23-47

Fig. 62



M 1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16 M 17 18 19 20 21 22 23 24 M

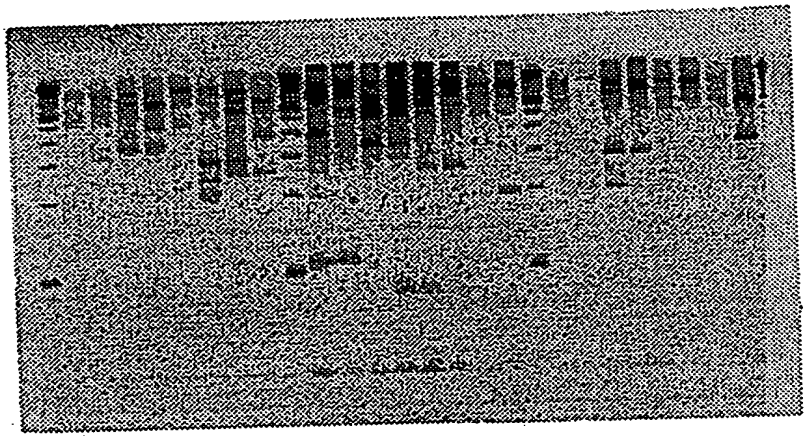
fraction number



M 25 26 27 28 29 30 31 32 M 33 34 35 36 37 38 39 40 M 41 42 43 44 45 46 47 48 M

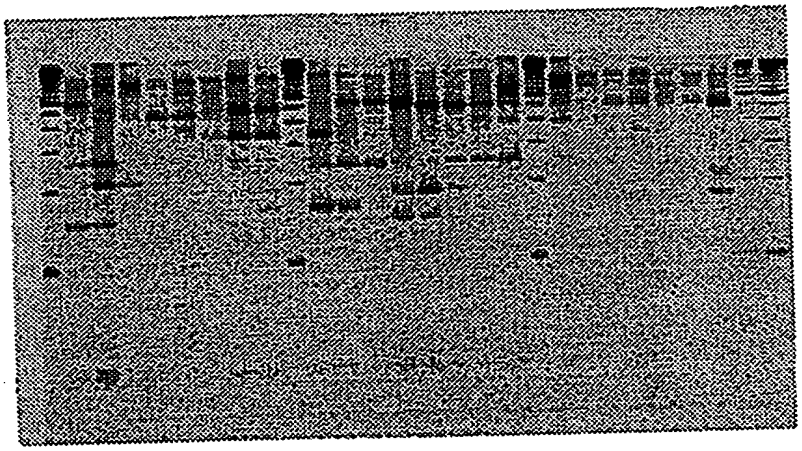
fraction number

Fig. 63



M 1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16 M 17 18 19 20 21 22 23 24 M

fraction number

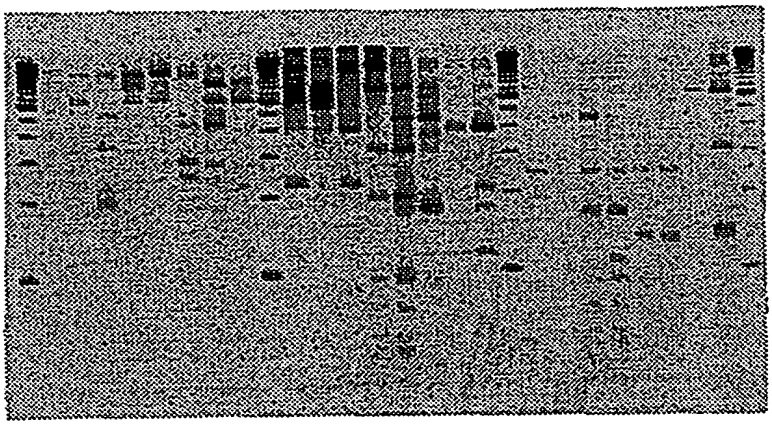


M 25 26 27 28 29 30 31 32 M 33 34 35 36 37 38 39 40 M 41 42 43 44 45 46 47 48 M

fraction number

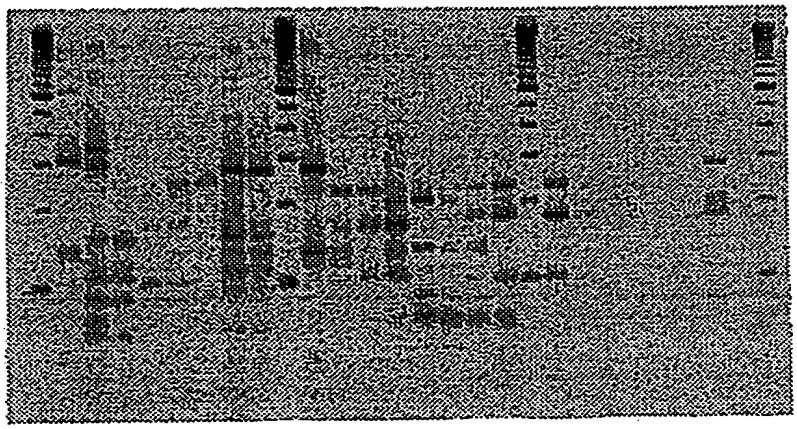
Fig. 64

88/1124



M 1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16 M 17 18 19 20 21 22 23 24 M

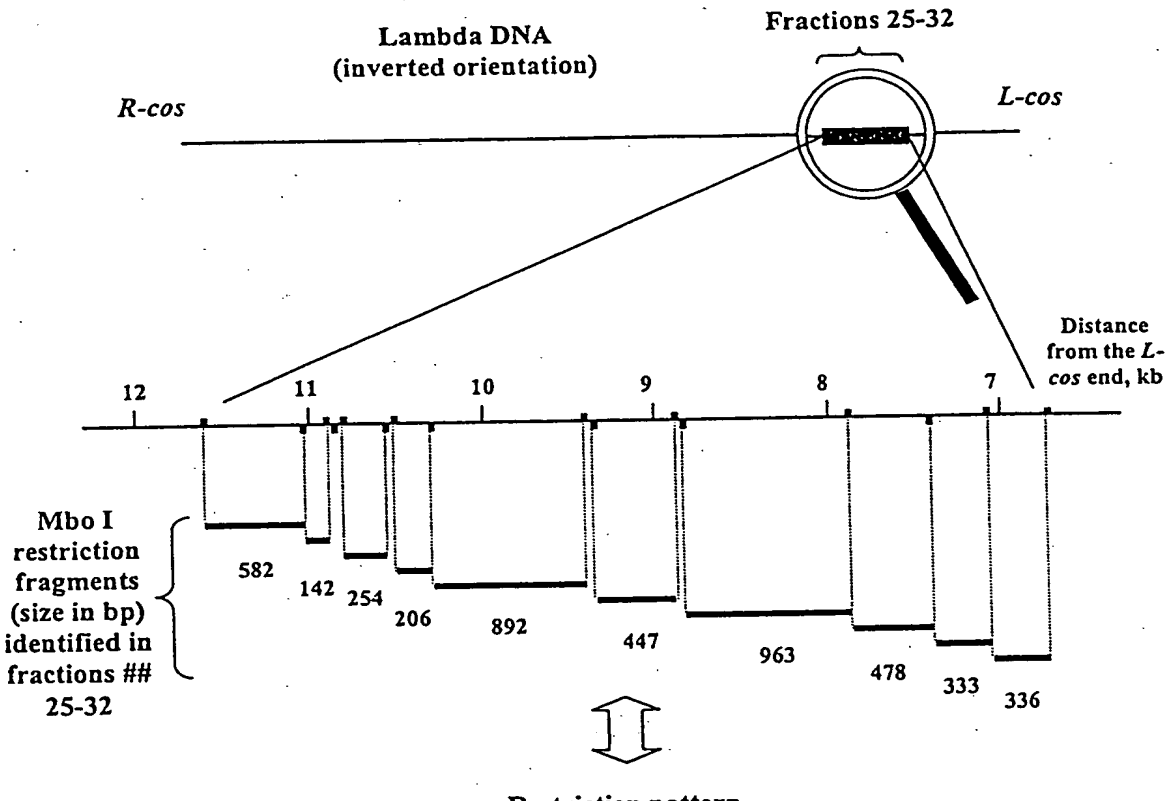
fraction number



M 25 26 27 28 29 30 31 32 M 33 34 35 36 37 38 39 40 M 41 42 43 44 45 46 47 48 M

fraction number

Fig. 65


Lambda DNA
(inverted orientation)
Fractions 25-32
R-cos
L-cos
Distance from the L-cos end, kb
12
11
10
9
8
7
Mbo I restriction fragments (size in bp) identified in fractions ## 25-32
582
142
254
206
892
447
963
478
333
336
Restriction pattern

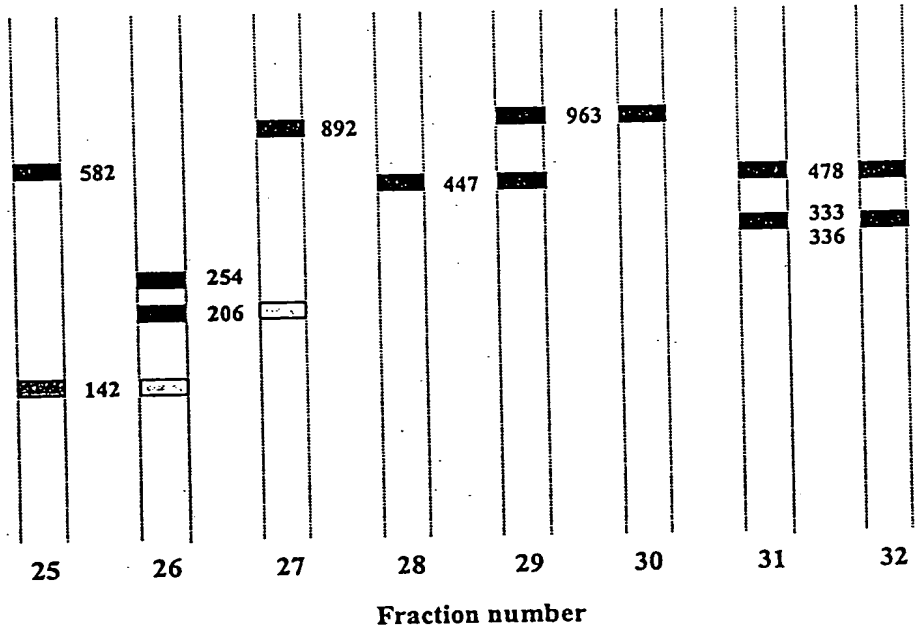

582
142
254
206
892
447
963
478
333
336
25
26
27
28
29
30
31
32
Fraction number

Fig. 66

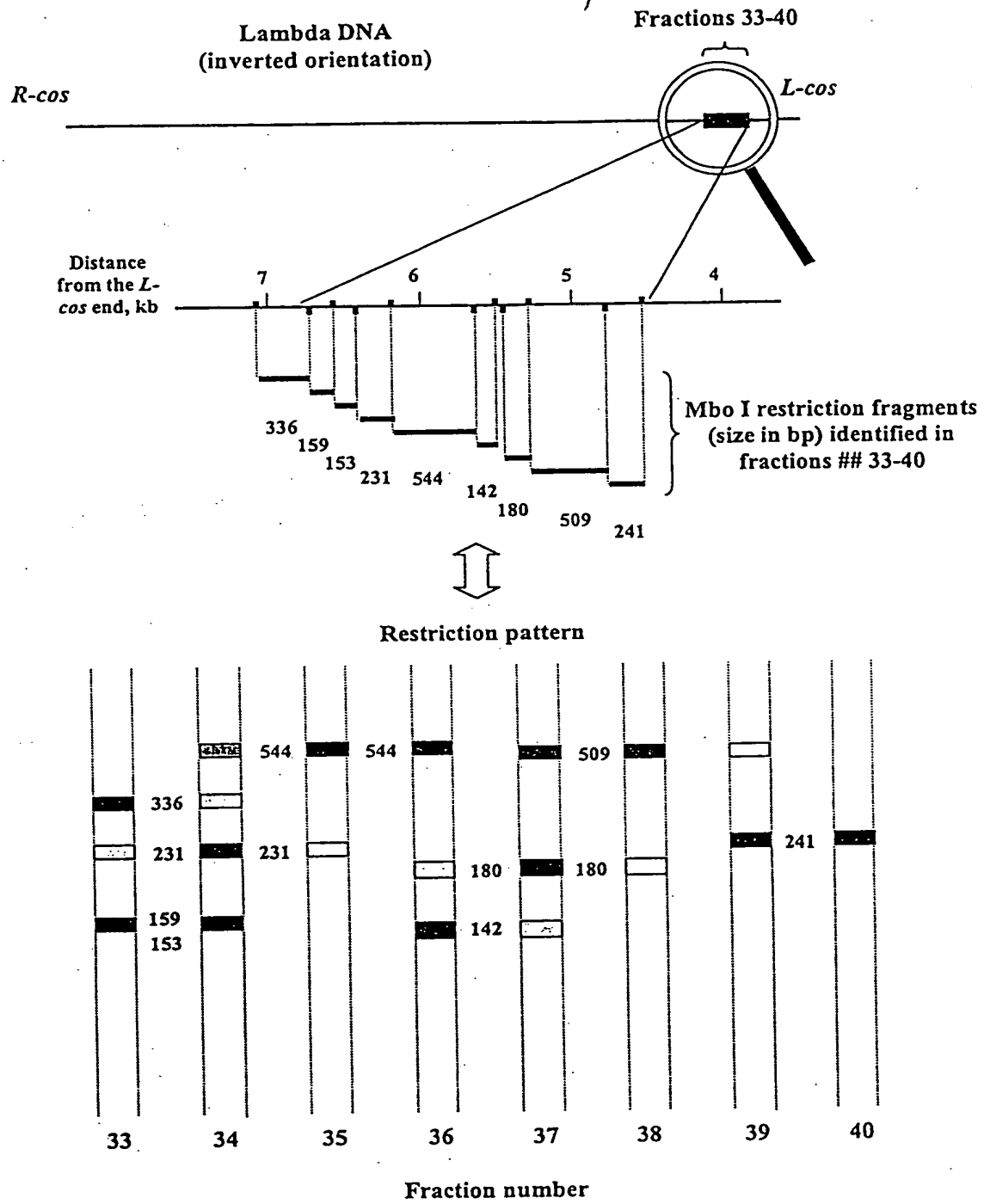

Lambda DNA
(inverted orientation)
Fractions 33-40
R-cos
L-cos
Distance from the L-cos end, kb
7
6
5
4
336
159
153
231
544
142
180
509
241
Mbo I restriction fragments (size in bp) identified in fractions ## 33-40
Restriction pattern
544
544
509
336
231
231
180
180
241
159
153
142
33
34
35
36
37
38
39
40
Fraction number

Fig. 67

FIG. 68

A) RA1/RA2(Methylation Dpn-I Activation)

RA1 5′-GATCTGAGGTTGTAGAAGACTCGGACGATACACATGCACCGTCGGTGCAGTCGTAATCCAGTCCCGA (SEQ ID NO:69) (SEQ ID NO:70 below)
AGCCACGTCAGCATTAGGTCAGGGCTCCTGTCGCGATAAAACGATCGGGCACTCGTTATTGATCGCTAG-5′ RA2

Assembled RA1 5′-(P)- GATCTGAGGTTGTAGAAGACTCGGACGATACACATGCACCGTCGGTGCAGTCGTAATCCAGTCCC**GATC**TCAGAGCGTT\
B3′-ACTCCAACATCTTCTGAGCCTGCTATGTGTACGTGGCAGCCACTGCAGCATTAGGTCAGGG**CTAG**AGTCTCGCTT/ (SEQ ID NO:71)

RA1 Component Oligos:
RA1(A) 5′-(P)- GATCTGAGGTTGTAGAAGACTCGGACGATACACATGCACCGTCGGTGCAGTCGTAATCCAGTCCC**GATC**TC-3′ (SEQ ID NO:72)
RA1(B) 5′-CTTCTACAACCTCA-B3′ (SEQ ID NO:73)
RA1(C) 5′-(P)-CGGTGCATGTGTATCGTCCGAGT-3′ (SEQ ID NO:74)
RA1(D) 5′-(P)-AGAGCGTTTTCGCTCTGAGATCGGGACTGGATTACGACTGCACCGA-B3′ (SEQ ID NO:75)

Assembled RA2 5′-(P)- GATCGCTAGTTATTGCTCACGGGCTAGCAAAATAGCGCTGTCCTCGGGACTGGATTACGACTGCACC**GATC**TCAGAGCG-T-T\
B3′-CGATCAATAACGAGTGCCCGATCGTTTTATCGCGACAGGAGCCCTGACCTAATGCTGACGTGG**CTAG**AGTCTCGC-T-T/ (SEQ ID NO:76)

RA2 Component Oligos:
RA2(A) 5′-(P)-GATCGCTAGTTATTGCTCACGGGCTAGCAAAATAGCGCTGTCCTCGGGACTGGATTACGACTGCACC**GATC**TC-3′ (SEQ ID NO:77)
RA2(B) 5′-GAGCAATACTAGC-B3′ (SEQ ID NO:78)
RA2(C) 5′-(P)-GGACAGCGCTATTTTGCTAGCCCGT-3′ (SEQ ID NO:79)
RA2(D) 5′-(P)-AGAGCGTTTTCGCTCTGAGATCGGTGCAGTCGTAATCCAGTCCCGA-B3′ (SEQ ID NO:80)

B) Simplified Recombinant Adapters Sra1/Sra2

Sra 1 5′**P**-GATCTGAGGTTGTTGAAGACTCGGACGATACACACGCTGGGTTGAGGAAGTCGTAAATA
TGTGCGACCCAACTCCTTCAGCATTTATTTATTGGTAGGGTTGTCGTTATTGATCGCTAG-5′**P** Sra 2

Sra 1A 5′**P**-GATCTGAGGTTGTTGAAGACTCGGACGATACACACGCTGGGTTGAGGAAGTCGTAAATA-3′ (SEQ ID NO:81)
Sra 1B B3′-ACTCCAACAACTTC-5′ (SEQ ID NO:82)
Sra 1C B3′-ACTCCAACAACTTCTGAGCCTGCT-5′ (SEQ ID NO:83)
Sra 1D B3′-TGTGCGACCCAACTCCTTCAGCATTTAT-5′ (SEQ ID NO:84)
3′-TGTGCGACCCAACTCCTTCAGCATTTATTTATTGGTAGGGTTGTCGTTATTGATCGCTAG-5′**P** Sra 2A (SEQ ID NO:85)
5′-CAGCAATAACTAGC-B3′ Sra 2B (SEQ ID NO:86)
5′-AACCATCCCAACAGCAATAACTAGC-B3′ Sra 2C (SEQ ID NO:87)
5′-ACACGCTGGGTTGAGGAAGTCGTAAATA-B3′ Sra 2D (SEQ ID NO:88)

C) Sra1′ Expanded complementarity with original Sra2

5′**P**-GATCTGAGGTTGTTGAAGACACGCTGGGTTGAGGAAGTCGTAAATAAATAACCATCCCAA
TGTGCGACCCAACTCCTTCAGCATTTATTTATTGGTAGGGTTGTCGTTATTGATCGCTAG-**P**5′

5′**P**-GATCTGAGGTTGTTGAAGACACGCTGGGTTGAGGAAGTCGTAAATAAATAACCATCCCAA-3′ Sra 1A′ (SEQ ID NO:89)
B3′-ACTCCAACAACTTC-5′ Sra 1B (SEQ ID NO:82)
B3′-TGTGCGACCCAACTCCTTCAGCATTTAT-5′ Sra 1D (SEQ ID NO:84)
B3′-TTATTGGTAGGGTT-5′ Sra 1H (SEQ ID NO:90)

FIG. 69

Simplified recombinant adapter (Sra) oligos

Sra 1A	P5'-GATCTGAGGTTGTTGAAGACTCGGACGATACACACGCTGGGTTGAGGAAGTCGTAAATA-3' (SEQ ID NO:91)
Sra 1B	5'-CTTCAACAACCTCA-B3' (SEQ ID NO:92)
Sra 1C	5'-TCGTCCGAGTCTTCAACAACCTCA-B3' (SEQ ID NO:93)
Sra 1D	5'-TATTTACGACTTCCTCAACCCAGCGTGT-B3' (SEQ ID NO:94)
Sra 2A	P5'-GATCGCTAGTTATTGCTGTTGGGATGGTTATTTATTTACGACTTCCTCAACCCAGCGTGT-3' (SEQ ID NO:95)
Sra 2B	5'-CAGCAATAACTAGC-B3' (SEQ ID NO:96)
Sra 2C	5'-AACCATCCCAACAGCAATAACTAGC-B3' (SEQ ID NO:97)
Sra 2D	5'-ACACGCTGGGTTGAGGAAGTCGTAAATA-B3' (SEQ ID NO:98)
Sra 1A'	P5'-GATCTGAGGTTGTTGAAGACACGCTGGGTTGAGGAAGTCGTAAATAAATAACCATCCCAA-3' (SEQ ID NO:99)
Sra 1H	5'-TTGGGATGGTTATT-B3' (SEQ ID NO:100)

Lambda recombination screening oligos

Total(+)	5'-AGGTTGTAGAAGACTCGG-3' (SEQ ID NO:101)
Total(-)	5'-GCTAGTTATTGCTCACGG-3' (SEQ ID NO:102)
Intra(+34273)	5'-GCATCGCTTGAATTGTCC-3' (SEQ ID NO:103)
Intra(-28119)	5'-TGCTCTCGGAATATCAAT-3' (SEQ ID NO:104)
Inter(+34273)	5'-GCATCGCTTGAATTGTCC-3' (SEQ ID NO:105)
Inter(-34599)	5'-ATATTCAGGCCAGTTATC-3' (SEQ ID NO:106)

E-coli recombination screening oligos

B1(RP)	5'-CTTACACCGGCGAAGTGAAAG-3' (SEQ ID NO:107)
B1(PCR)	5'-CGCTGCCGGAGCTGTTAGACAATTC-3' (SEQ ID NO:108)
B1(NEST)	5'-GCCTGCAAGCCGGTGTAGACATCAC-3' (SEQ ID NO:109)
B3(RP)	5'-CTGCAGGCCAGCGAGACAGAT-3' (SEQ ID NO:110)
B3(PCR)	5'-GTTGTGGCCTTCCAGTAAGGTCC-3' (SEQ ID NO:111)
B3(NEST)	5'-GCAAAATAGCTGGCTGGCAGGTGTAGG-3' (SEQ ID NO:112)
B5(RP)	5'-TAGGGCGGCATCAGGTAATAC-3' (SEQ ID NO:113)
B5(PCR)	5'-TGCCGCCGTTCGCATCCATACCA-3' (SEQ ID NO:114)
B5(NEST)	5'-TTCCCTGCCTGGTCGCCGTATCTGTG-3' (SEQ ID NO:115)
B8(RP)	5'-TGAAGGATACGGAAGCAGAAA-3' (SEQ ID NO:116)
B8(PCR)	5'-GCCATTGCTGATTGCCCACCGACAA-3' (SEQ ID NO:117)
B8(NEST)	5'-CTCTATCGCTCGGCCTAAGTCTTTAC-3' (SEQ ID NO:118)
B12(RP)	5'-GCGGTCGGCGTGGATAAAGTA-3' (SEQ ID NO:119)
B12(PCR)	5'-GTGAGCGGGATGAACGAACCTTA-3' (SEQ ID NO:120)
B12(NEST)	5'-CTGCGCCAGGGCTTCCAGACATTGTG-3' (SEQ ID NO:121)

FIG. 70


Inter-molecular junctions
Intra-molecular junctions
total junctions
uncut λ
1:1000
1:100
1:10
100bp
Control
uncut λ
1:1000
1:100
1:10
100bp
Control
uncut λ
1:1000
1:100
1:10

FIG. 71

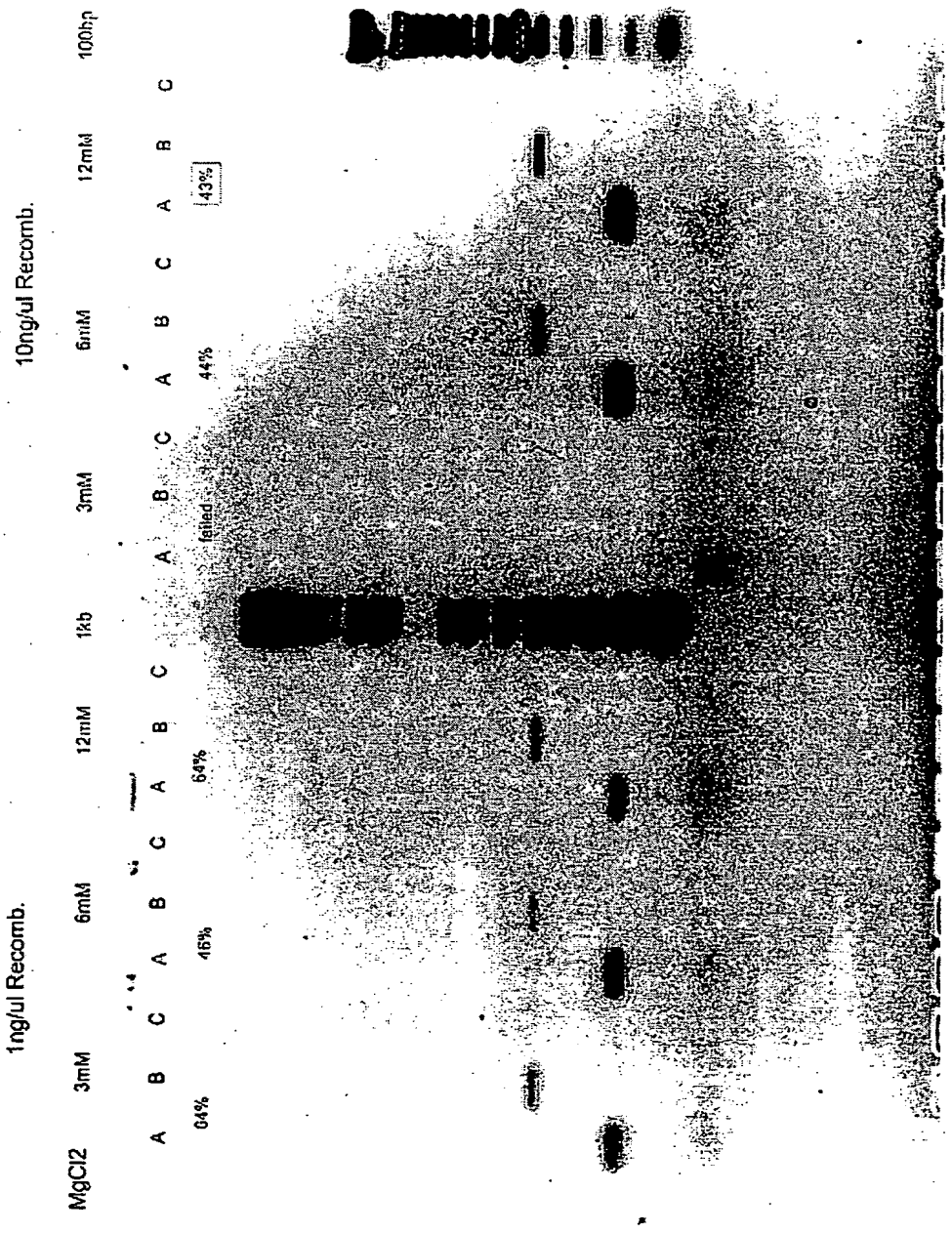

1ng/ul Recomb.
10ng/ul Recomb.
MgCl2
3mM
6mM
12mM
1kb
100bp
A B C A B C A B C A B C A B C
04%
16%
64%
failed
44%
43%

FIG. 72

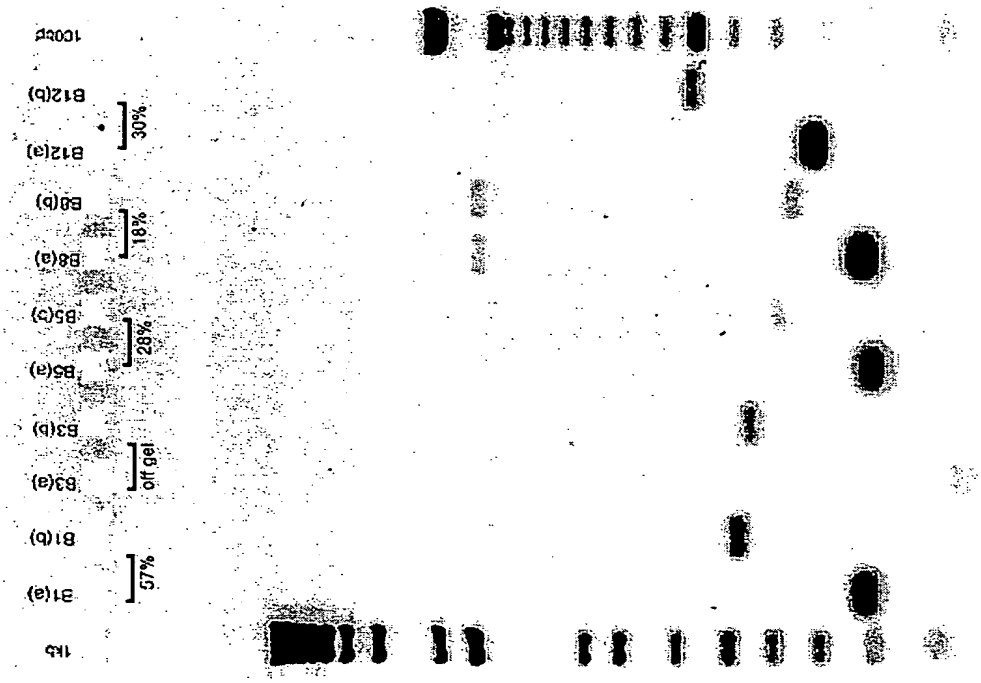

1kb
31(a)
57%
31(b)
53(a)
off gel
53(b)
56(a)
28%
55(b)
58(a)
18%
58(b)
312(a)
30%
312(b)
1000bp

FIG. 73

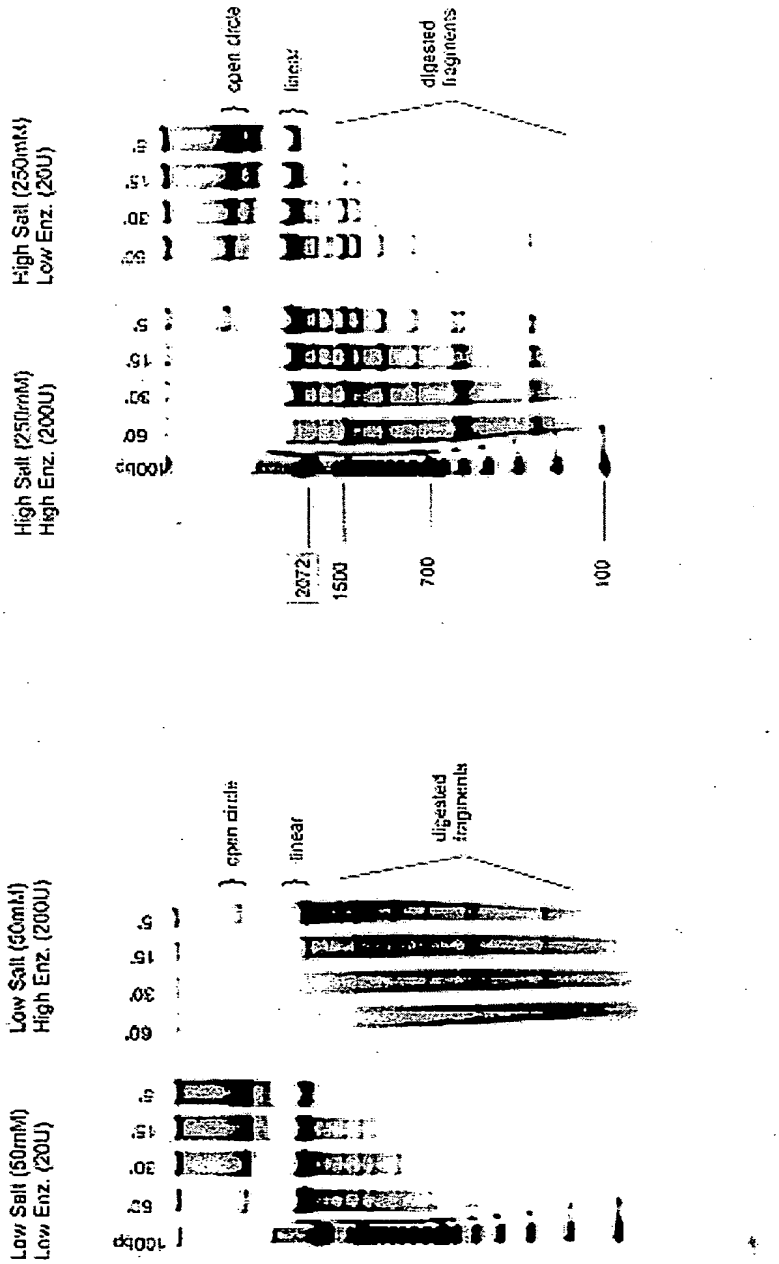

High Salt (250mM)
Low Enz. (20U)
High Salt (250mM)
High Enz. (200U)
Low Salt (50mM)
High Enz. (200U)
Low Salt (50mM)
Low Enz. (20U)
open circle
linear
digested fragments
5'
15'
30'
60'
100bp
2072
1500
700
100

FIG. 74

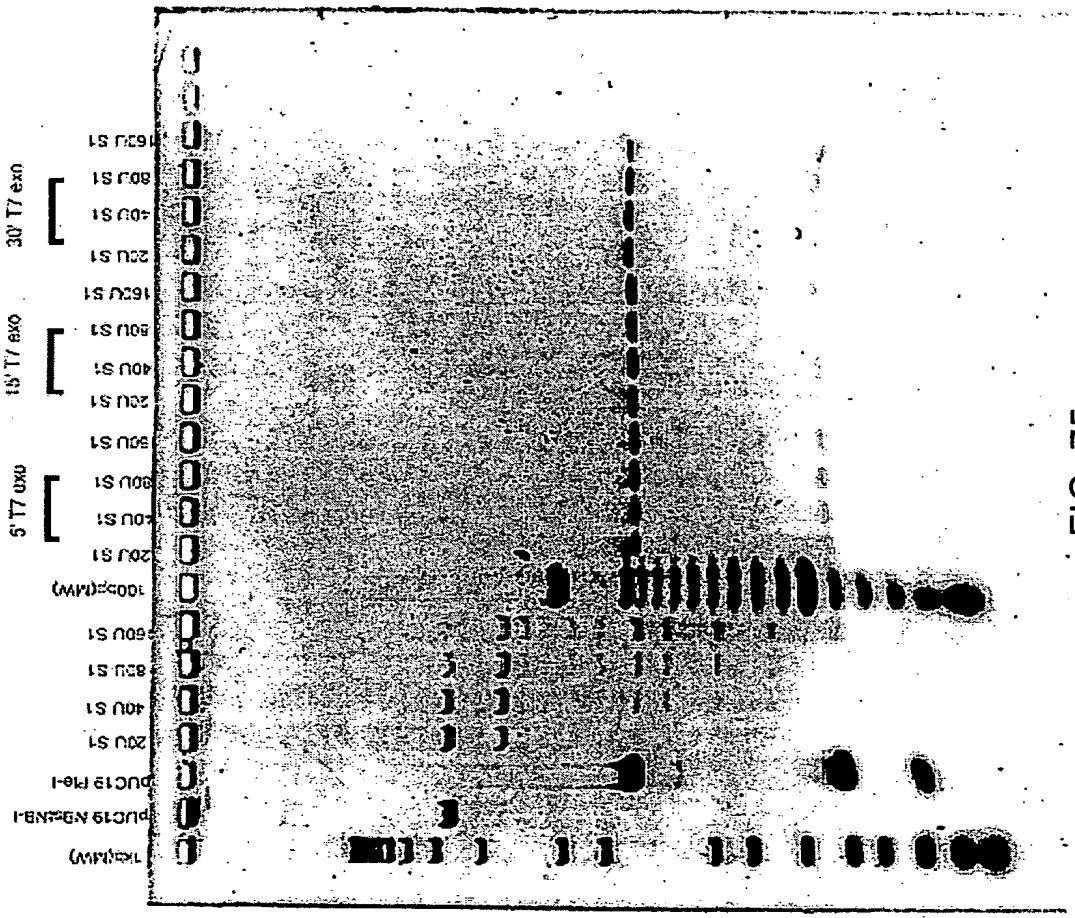

5' T7 exo
15' T7 exo
30' T7 exo
20U S1
40U S1
80U S1
160U S1
20U S1
40U S1
80U S1
160U S1
20U S1
40U S1
80U S1
160U S1
pUC19 MspI
pUC19 NspBII
Marker

FIG. 75

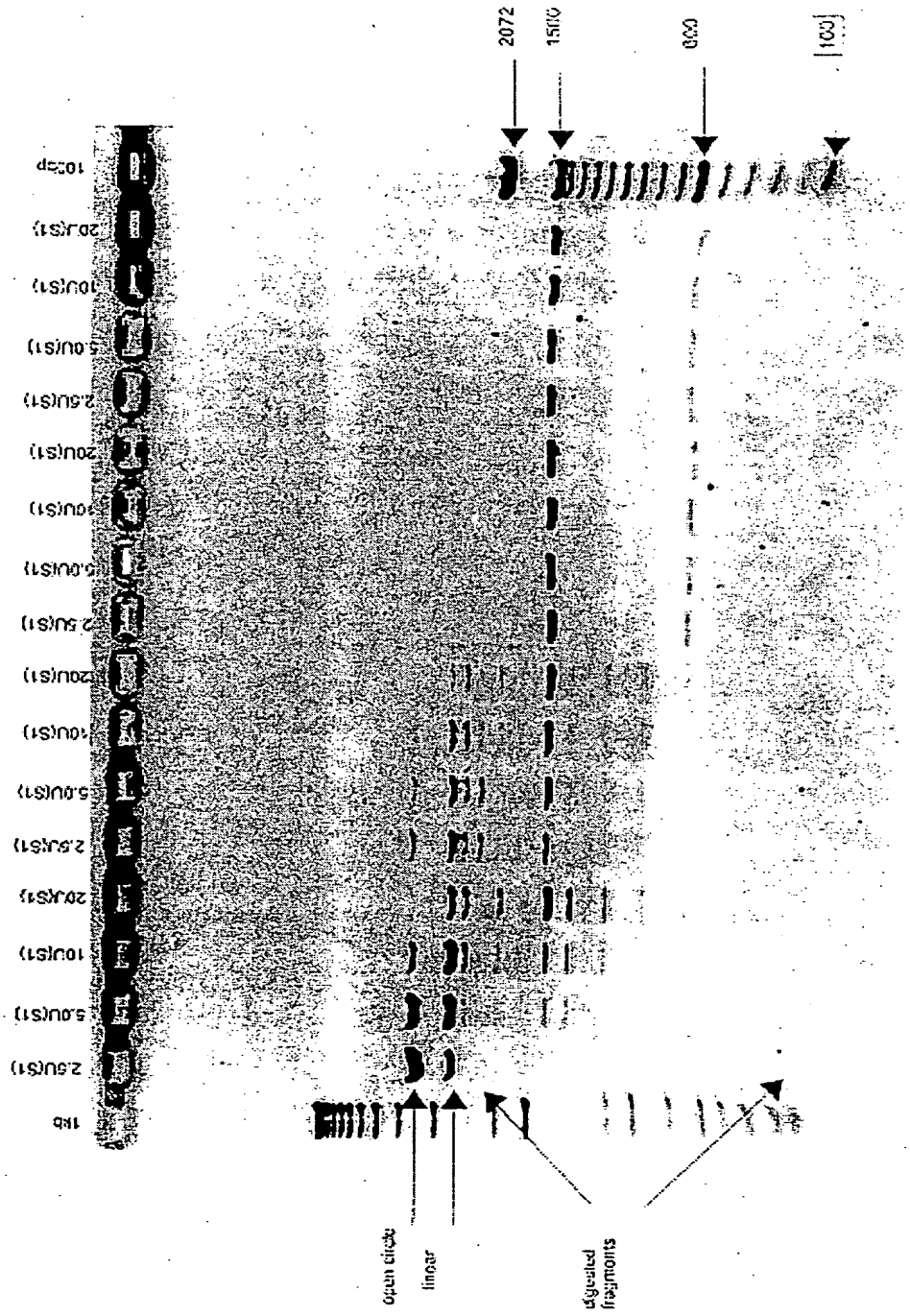


FIG. 76

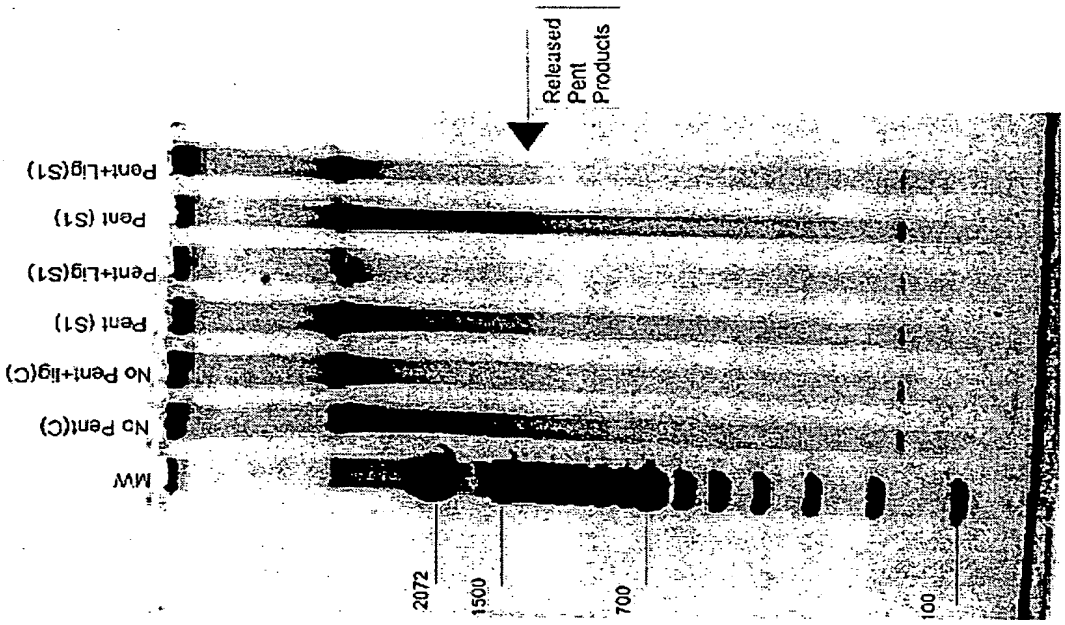

Released Pent Products
Pent+Lig(S1)
Pent (S1)
Pent+Lig(S1)
Pent (S1)
No Pent+lig(C)
No Pent(C)
MW
2072
1500
700
100

FIG. 77

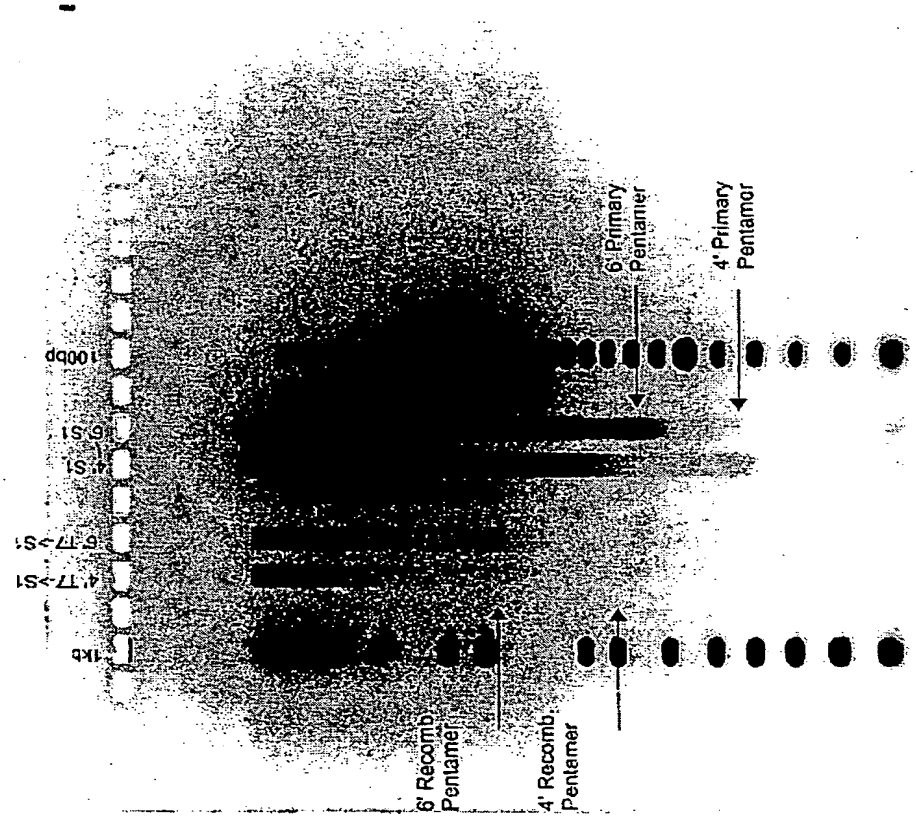
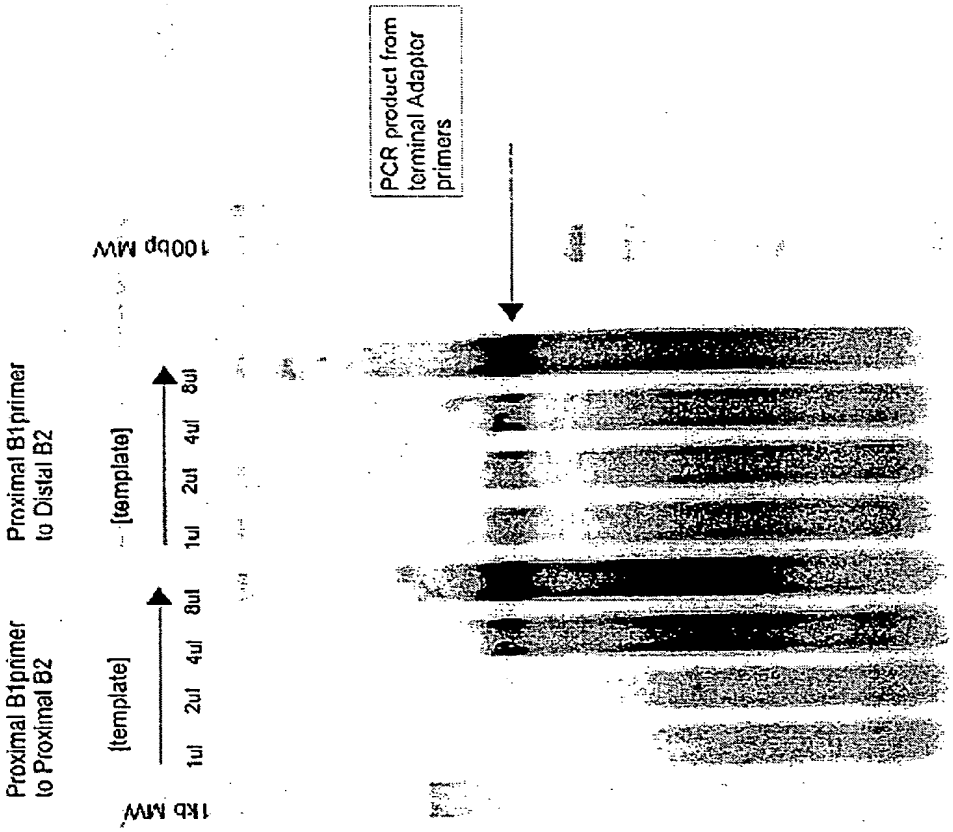

100bp
6'S1
4'S1
6'T7>S1
4'T7>S1
1kb
6' Recomb. Pentamer
4' Recomb. Pentamer
6' Primary Pentamer
4' Primary Pentamer
Enzymatic Release of Recombinant Pentamers T7 gene6 - S1 nuclease

FIG. 78



Amplification of Secondary Nick Translation released recombinant Pentamers

FIG. 79

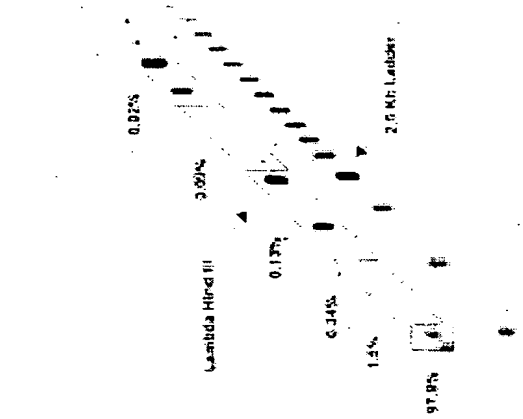


FIG. 80B

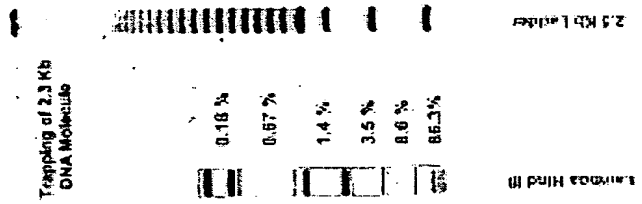


FIG. 80A

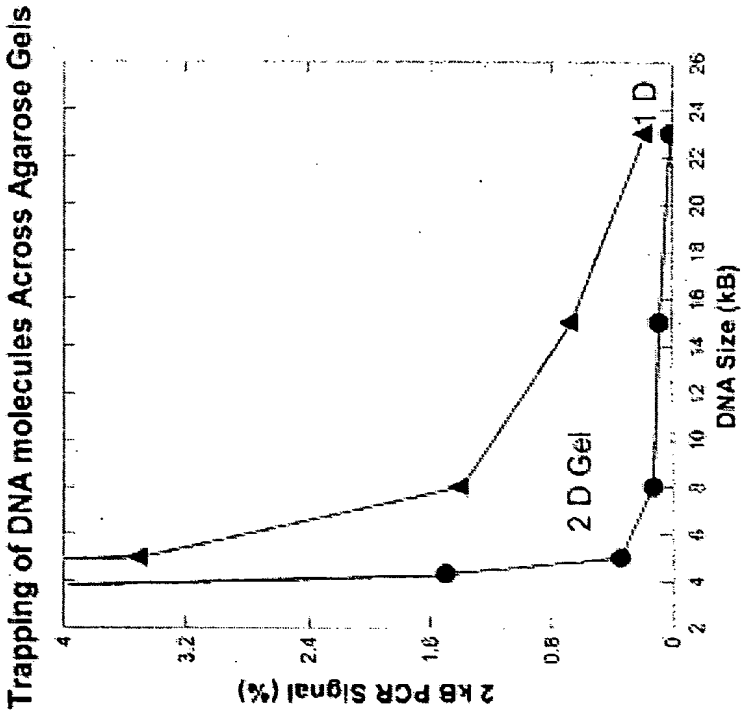

Trapping of DNA molecules Across Agarose Gels
2 kB PCR Signal (%)
DNA Size (kB)
2 D Gel
1 D
0
0.8
1.6
2.4
3.2
4
2
4
6
8
10
12
14
16
18
20
22
24
26

FIG. 81

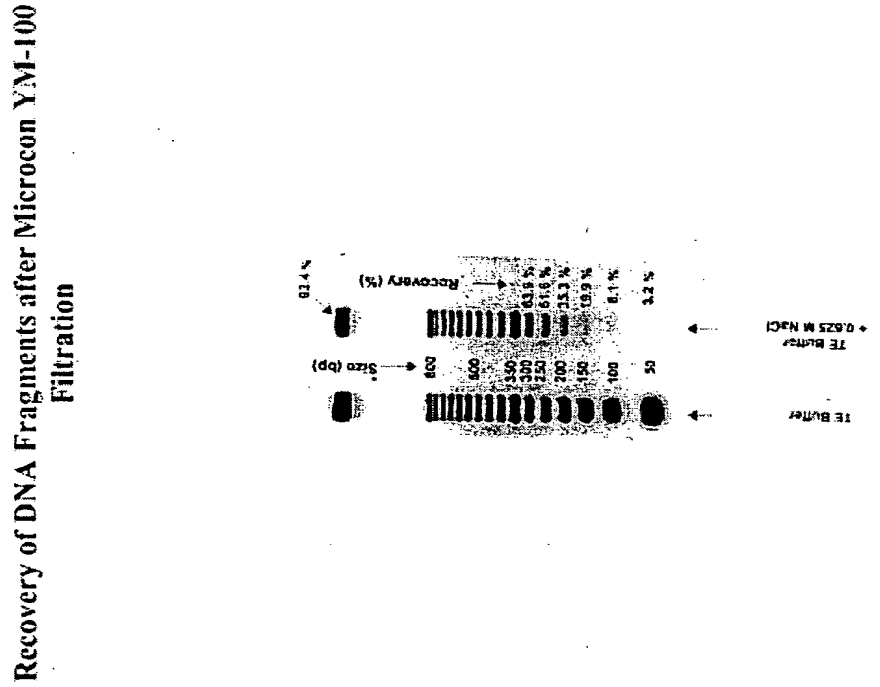

Recovery of DNA Fragments after Microcon YM-100 Filtration
Recovery (%)
Size (bp)
TE Buffer
TE Buffer + 0.625 M NaCl

FIG. 82

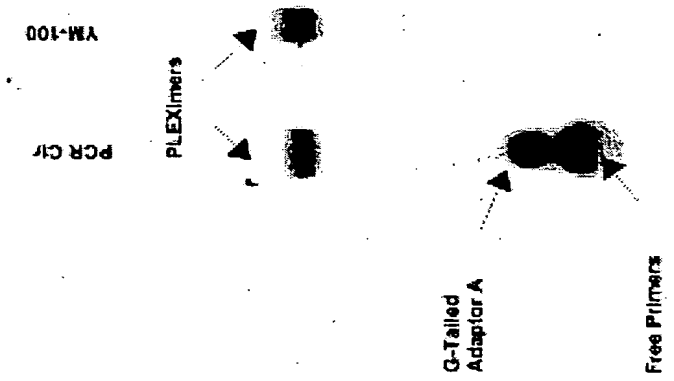


FIG. 83

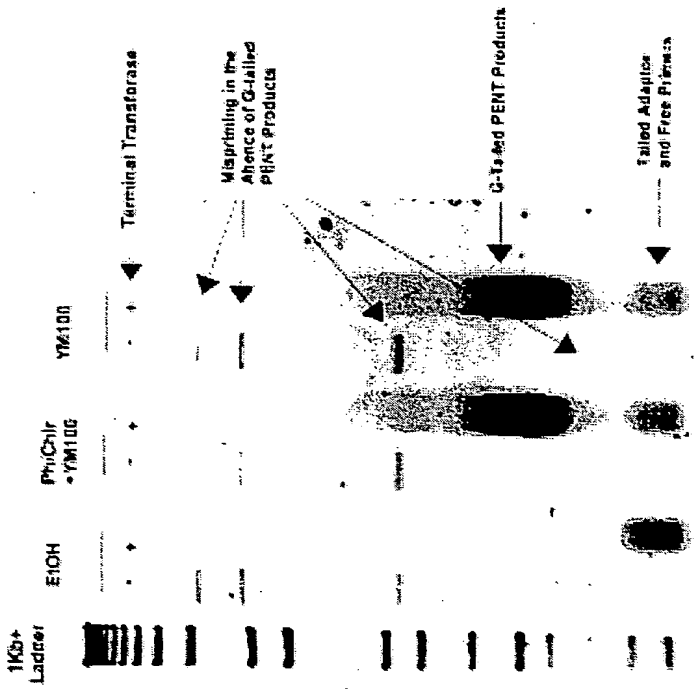

1Kb+ Ladder
EtOH
PhiChlr +YM100
YM100
Terminal Transferase
Misprimimg in the Absence of G-tailed PENT Products
G-Tailed PENT Products
Tailed Adaptor and Free Primers

FIG. 84

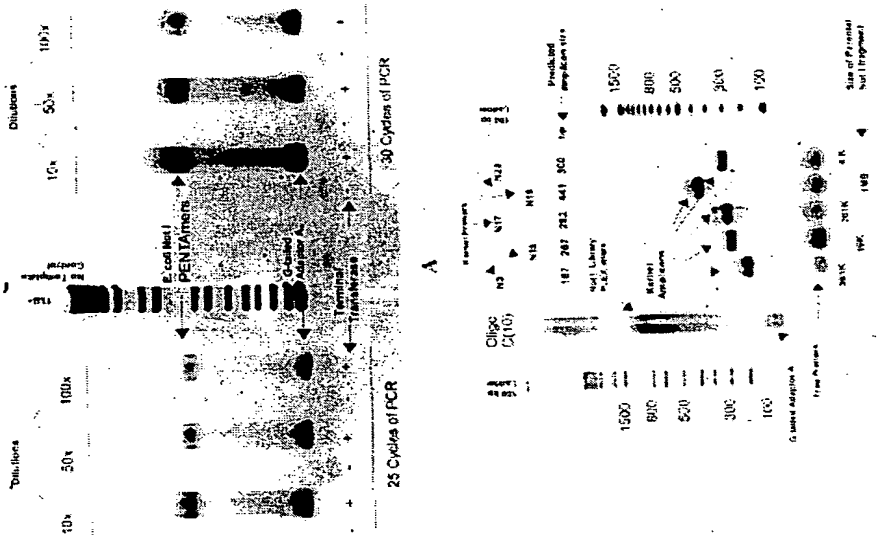

A
B
25 Cycles of PCR
30 Cycles of PCR
Dilutions
10x
50x
100x
E. coli Nat I
PENTAmers
G-tailed Adaptor A
Terminal Transferase
Oligo dC(10)
Nat I Library PCR Amplicons
Predicted amplicon size
Kanrad Amplicons
1500
800
500
300
100
Size of Parental Nat I Fragment
48K
261K
194K

FIG. 85

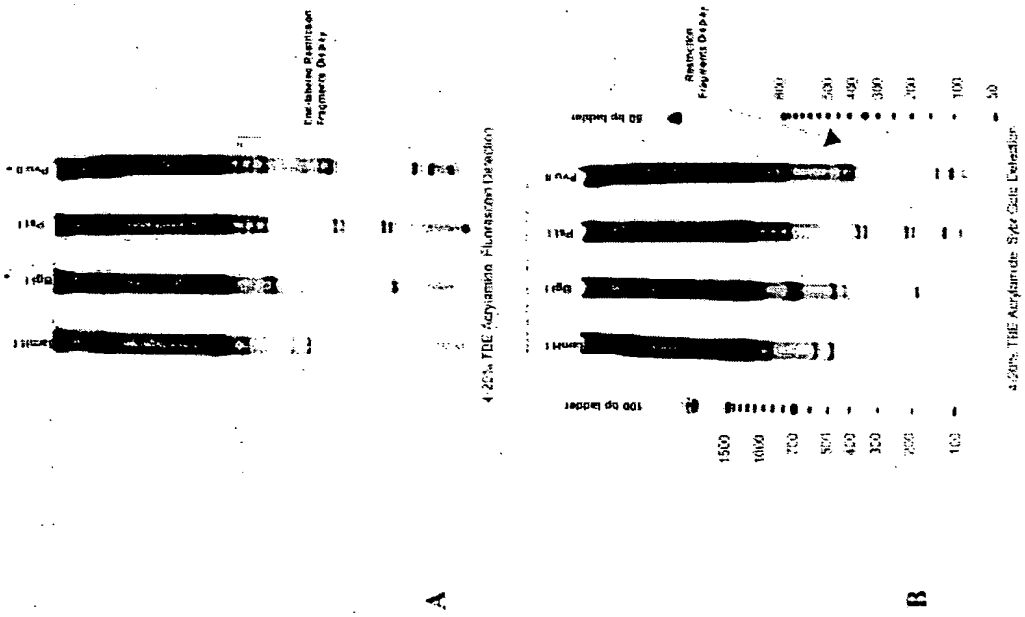


FIG. 86

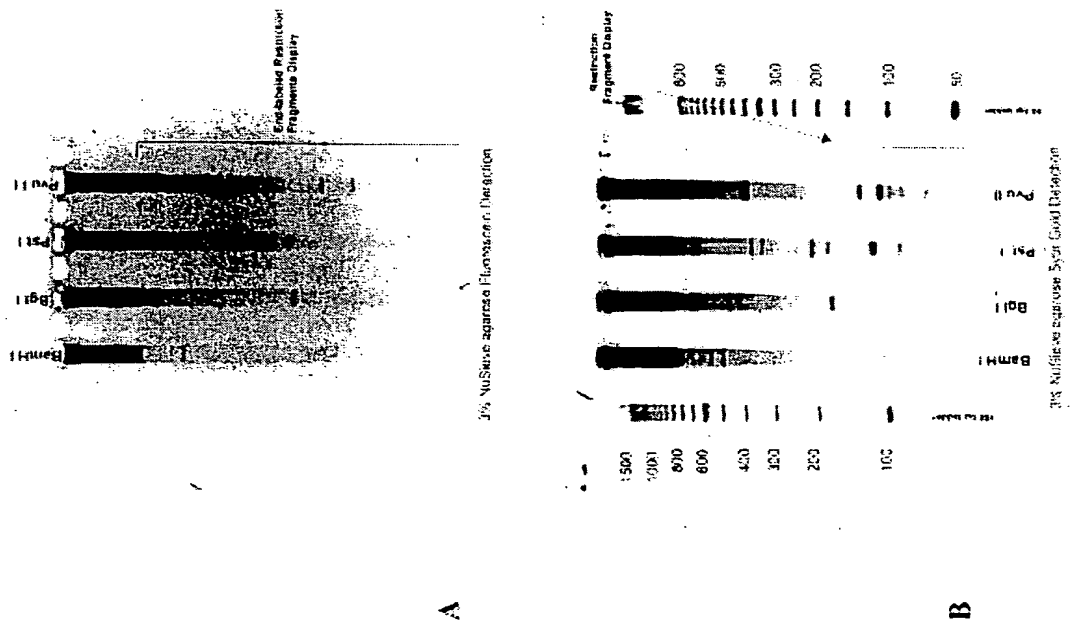

A
B
BamH I
Bgl I
Pst I
Pvu II
1500
1000
800
600
400
300
250
100
850
650
300
200
150
50

FIG. 87


Library PENTAmers
Adaptor A-B Dimers
Number of PCR Cycles
18
21
23
26
BamHI Library
Sau3A Library

FIG. 88

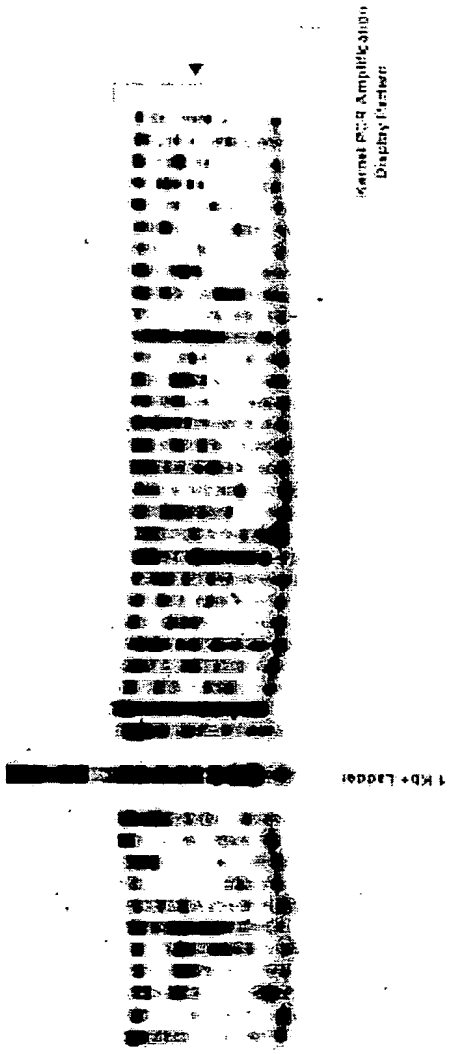


FIG. 89

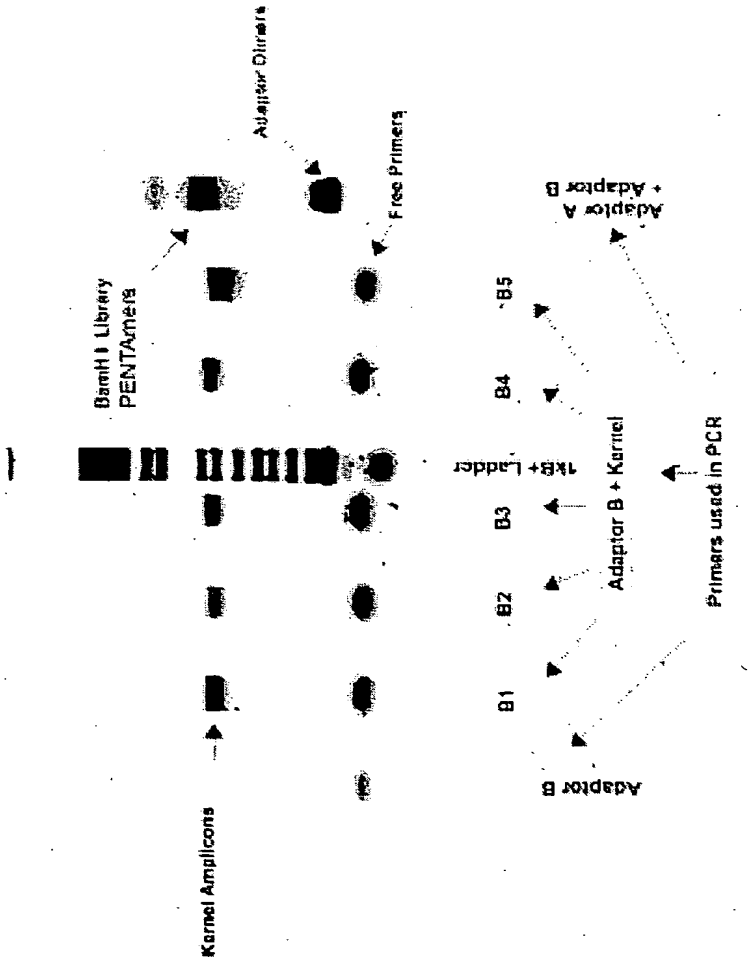

Adaptor Dimers
Free Primers
BamH I Library
PENTAmers
Kernel Amplicons
1kB+ Ladder
B5
B4
B3
B2
B1
Adaptor A
+ Adaptor B
Adaptor B + Kernel
Primers used in PCR
Adaptor B

FIG. 90

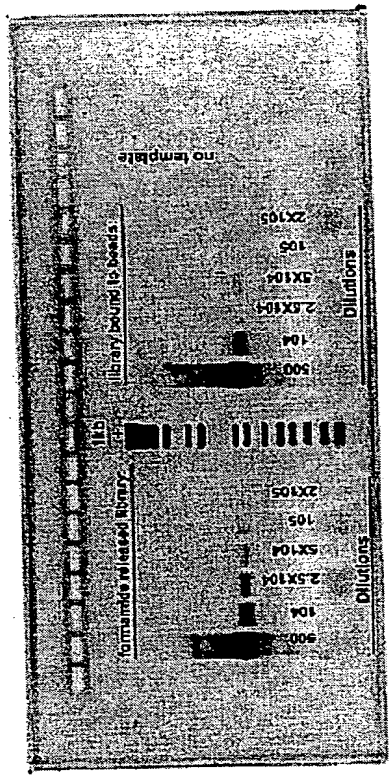

A
1kb
formamide released library
library bound to beads
no template
Dilutions
2X10^5
10^5
5X10^4
2.5X10^4
10^4
500
B
no template
1kb
Single-stranded library bound to beads:
Single-stranded library formamide released:
Double-stranded library formamide released:
Dilutions
5X10^3
2X10^3
10^3
500
250
50
5X10^4
10^4
105

FIG. 91